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Executive Summary: Ames Research Center

Ames Research Center NAI team has maintained a coordinated research program that links the formation, evolution, and climates of habitable planets; the roles of interstellar chemistry in supplying potential biological precursors to these worlds; the origins and nature of metabolism in the first cells; the impact of established biospheres on planetary climate and crustal and atmospheric chemistry, emphasizing the formation of detectable biosignatures; the response of vegetation to regional climate change; and, finally, the potential for life to transcend planetary boundaries through transfer between habitable worlds. Our program for education and public outreach captures these themes to develop an engaging and informative package that is being disseminated to national- and international-scale audiences. This is being achieved through partnerships with the California Academy of Sciences (CAS), Yellowstone National Park (YNP), New York Hall of Science, and several K-14 educational organizations. Strong conceptual and functional links to multiple NASA missions provide context, motivation, and funding leverage for our research component, along with resource- and audience-sharing opportunities for our education and public outreach component.

Our investigations of the formation, evolution, and climatology of habitable planets have focused on terrestrial (rocky) planets where liquid water is stable at the surface. We chose this focus because extrasolar planets that host surface biospheres are the most likely to be detected by remote spectroscopic searches. Simulations of planetary accretion have shown that the amount of mass accreted by Earth from the asteroid region depends sensitively on the formation time and the early orbital evolution of the giant planets. In particular, the mass of volatile materials delivered to Earth depends critically on the orbital eccentricities of giant planets, because these control the width and strength of the unstable resonances in the asteroid region. Our team has shown that methane could have provided a maximum of 30 degrees C of surface warming on early Mars. Higher methane concentrations would have resulted in an anti-greenhouse effect that would have caused surface cooling. We proposed a novel explanation for the Martian rivers, namely that they originated as the result of impacts. This view greatly restricts the environments where life might have originated and suggests that the only likely place might be in the subsurface. For the first time, we created particles that may form in methane-rich atmospheres that also contain carbon dioxide. Such atmospheres might resemble those of early Earth.

We have studied the formation of complex and potentially protobiological organics under simulated interstellar conditions. We also have helped to trace, spectroscopically and chemically, the cosmic evolution of organic molecules from the interstellar medium to protoplanetary disks, planetesimals, and finally onto habitable bodies. We have made progress in tracing the link between ice processes and the organic molecules in meteorites.

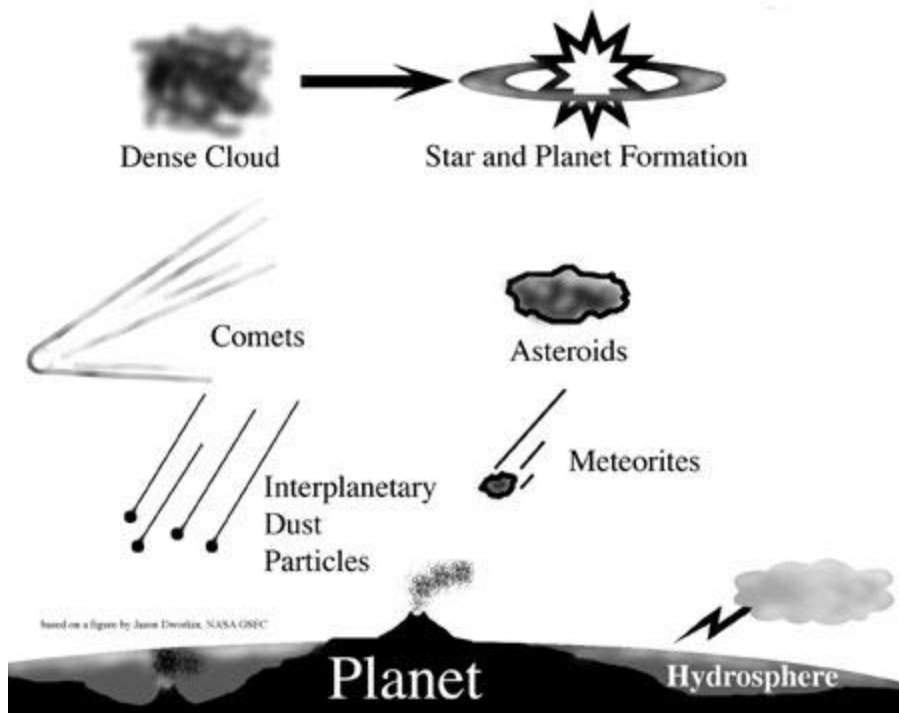


Figure 1. *Results from this past year's effort support the hypothesis that molecules that are important for the origins of life, might originate in the interstellar medium.*

First, we have demonstrated that the ultraviolet (UV) photolysis of presolar ices can produce the amino acids alanine, serine, and glycine, as well as hydroxy acids, and glycerol, all of which have been extracted from the Murchison meteorite. Thus, some of the meteoritic amino acids glycine, alanine and serine, as well as structurally related hydroxy acids, may have been synthesized in ice before our solar system formed. Also, the amino (NH₂), carboxylic acid (COOH), cyano (CN), ether (O-CH₃), hydroxy (OH), keto (>C=O), and methyl (CH₃) groups that decorate the aromatic compounds in meteorites and interplanetary dust particles (IDPs) can be formed by energetic processing of low temperature ices. This pathway is consistent with observed deuterium enrichments and is the first proposed mechanism that makes predictions on a molecular level.

We also address the origin of metabolism in the earliest ancestors of cells by testing the hypothesis that proteins might have arisen and initially evolved in the absence of a genome. We selected four new adenosine triphosphate (ATP)-binding proteins from a population of $>10^{12}$ random polypeptides. Proteins from one family have been characterized. They are highly selective and do not bind either guanosine triphosphate (GTP) or deoxyadenosine triphosphate (dATP). They do not contain any known ATP-binding motifs, but they do require zinc ions and contain conserved cysteine residues, suggesting a possible structural similarity to zinc finger proteins. In order to test this possibility, and more generally to determine if these de novo evolved proteins resemble biological protein, we have evolved mutants that are suitable for biophysical studies by continued selection for ATP binding in the presence of guanidine. As expected, more of the newly selected proteins stay soluble in the presence of ATP, presumably because ATP binding stabilizes the folded state of the protein. We have established that some proteins remain stable as monomers. To simulate self-organization of protobiological proteins into metabolic networks capable of evolution towards increasing complexity we developed a computational approach for describing systems having many species and reaction channels. This method is not limited to chemical reactions, and it allows for incorporation of other cellular processes such as channel-mediated transport and cell growth and division.

We investigated living photosynthetic microbial mat ecosystems because they allow us to examine those microbiota and ecological processes that participated in early evolution, modified the early environment, and created biosignatures. Photosynthetically active cyanobacterial mats were studied in a hypersaline pond near Guerrero Negro, Mexico. We quantified the substantial quantities of volatile fatty acids (VFA) produced by these mats. We measured fluxes of dimethylsulfide (DMS) and methanethiol (MT) under a range of conditions. These two gases are probably formed by reactions that occur between low molecular weight organic carbon compounds and biogenic hydrogen sulfide. In an early anoxic global environment, DMS and MT can escape to the atmosphere and serve as biosignatures of these microbial communities. Our microscopic, genetic, biomarker and biogeochemical observations indicated that hypersaline mats can be maintained in a greenhouse at Ames for more than a year without sustaining major changes. This greenhouse will allow astrobiologists to explore the microbiological effects of environmental conditions that were widespread on early Earth but that are rare today. We documented a high diversity of uncultivated green nonsulfur bacteria (GNS) in photosynthetic microbial mats in the hot, alkaline Mushroom Spring, Yellowstone National Park. Some GNS were observed in close association with cyanobacteria, and diverse members of the GNS group assimilated acetate.

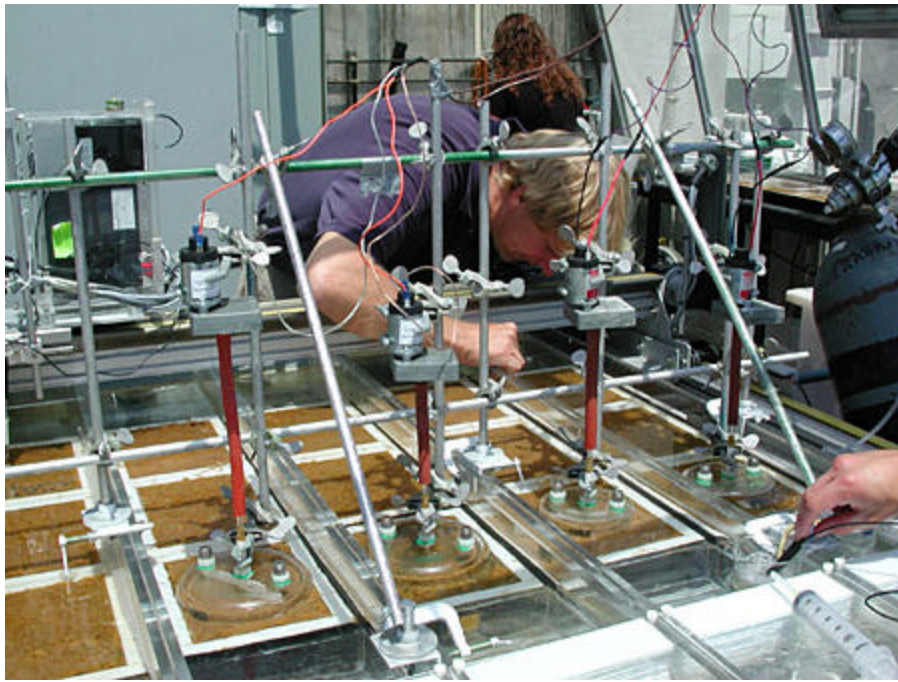


Figure 2. *Recent experiments indicate that the Ames greenhouse facility enables us to study microbial mats under environmental conditions that were important on early Earth but are either rare or absent today.*

We are extending the ecosystem-level studies of photosynthetic microbial mats to a planetary scale by creating quantitative models that simulate energy relationships, biogeochemical cycling, trace gas exchange, and biodiversity. We developed a simulation model called MBGC (Microbial BioGeoChemistry) to infer effects of major environmental controllers on microbial community structure and function. Already the model reproduces major diel fluctuations in trace gases in hypersaline subtidal mat layers and into the overlying water column. We are testing various approaches to simulating the net hourly emissions of O₂, CO₂, and H₂S gases to the atmosphere above the mat ecosystem, over the course of several days during which actual measurements of these same fluxes have been made.

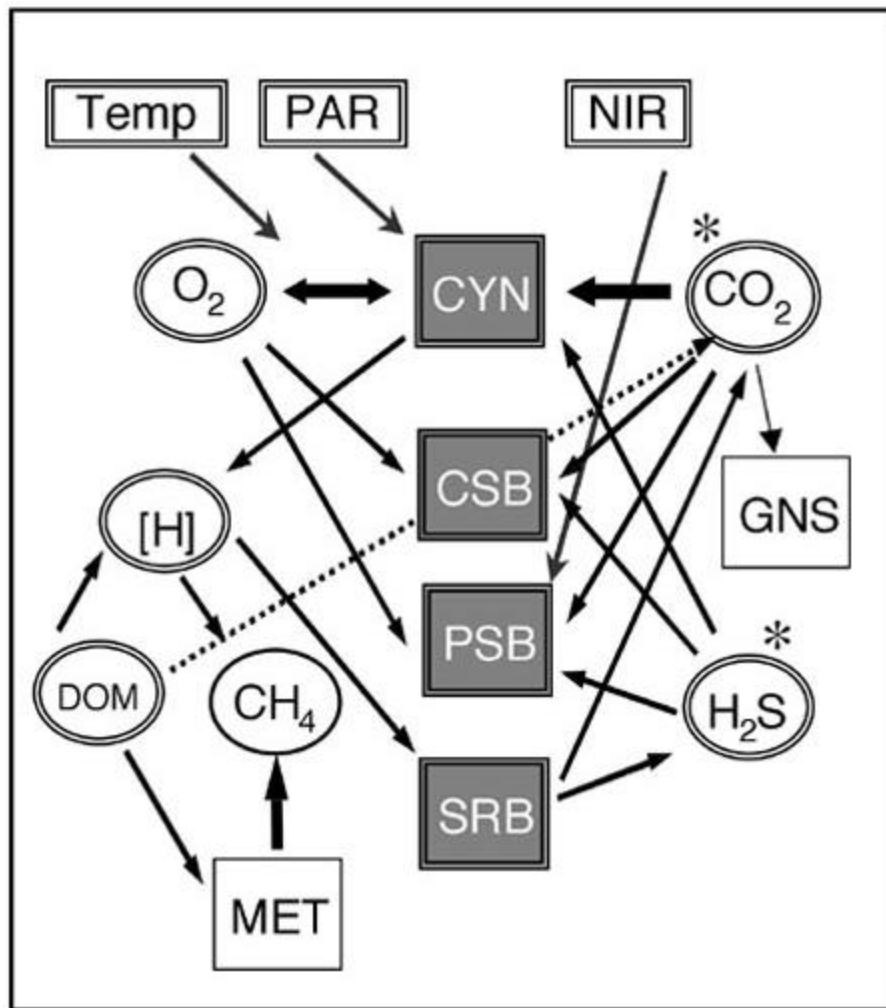


Figure 3. Schematic representation of the MBGC model (Decker and Potter, 2002), including bacterial pools (squares), light (PAR, NIR) and temperature input as model drivers, and gas concentration pools (circles).

We have developed, for community use, a new Global Atmospheric Integrator for Astrobiology (GAIA). It is unique in that it can consistently leap from simulations of a "typical" day of varying atmospheric chemistry to periods of geologic time. GAIA incorporates fully modern descriptions of atmospheric photolysis and UV shielding, photochemical reactions, thermal reactions, a simple water cycle, and lower and upper driving fluxes, i.e., biogeochemical interchange and hydrogen escape. GAIA performs stably as fluxes of O₂ and reduced (CH₄, H₂) gases are increased, thus allowing a full study of composition accompanying the rise of aerobic life.

We are examining the effects of climate variability on a vegetation-rich biosphere over intermediate time scales, using South American ecosystems as a model. Previously, we demonstrated that a strong correlation exists between vegetation changes at 32 South American sites and variations in sea-surface temperature (SST) over a 12-year period, related to the El Niño Southern Oscillation (ENSO). This past year, our map of remote-sensing-based estimates of vegetation indices (advanced very-high-resolution radiometer - normalized difference vegetation index (AVHRR-NDVI)) for the subtropical growing season of South America (October through January) on monthly data from 1981 through 2000 validates earlier ground-level studies by other investigators. Our graphic model also has modified current knowledge regarding the areas affected by "El Niño" Southern Oscillation in South America. We identified areas that are affected differently by El Niño, and La Niña, as well as the "normal" conditions. We should eventually be able to link SST to tree-ring widths. Finally, our reconstructed "paleo"-SST simulation correlates well with volcanic phenomena, the Little Ice Age, and other events. The simulation also complements, often with greater detail, reconstructions made with other proxy records, such as paleomicroplankton.

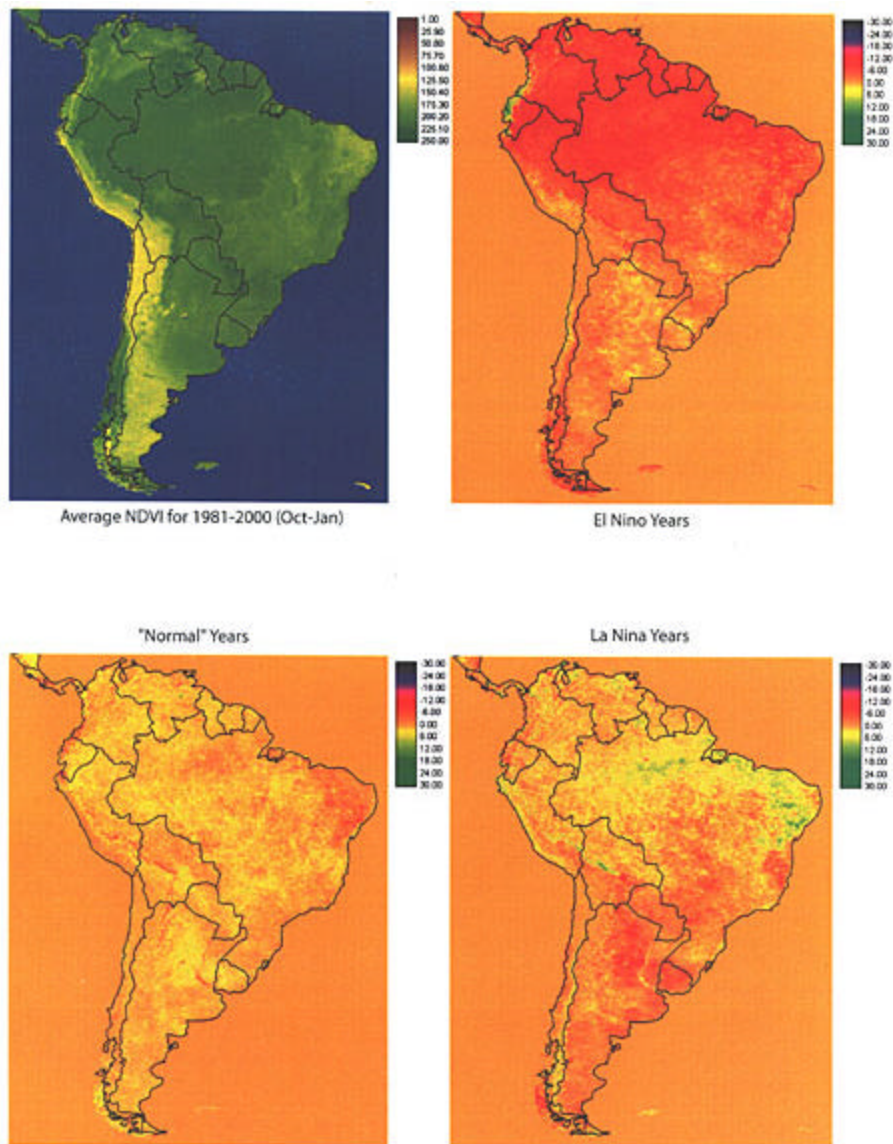


Figure 4. Summary description of South America's AVHRR-NDVI-deviations from normal (1981-2000) under "El Niño", and "La Niña" conditions.

We are assessing the potential for life to move beyond its planet of origin, as a potentially important component in the evolution of life in our own solar system. We address natural transport where survivors must withstand radiation, desiccation, and time in transit. Observations both in the laboratory and in nature indicate that low levels of radiation and oxidative damage on cells can result in a mitogenic effect, meaning that responses to radiation are non-linear and cannot be predicted from simple models. Experiments conducted in the space simulation facility at the Deutsche Forschungsanstalt für Luft- und Raumfahrt (DLR) in Cologne, Germany reveal that the cyanobacterium *Lyngbya* can survive exposure to both UV radiation and desiccation. The number of organisms that might be able to survive exposure to the space environment might be far greater than originally recognized.

The strong mission relevance of our research, combined with the direct participation of several team members in the planning and execution of NASA missions, places the Ames team in a position to influence strongly the astrobiology content of ongoing and future missions. Studies of planet formation and habitability currently benefit the Space Infrared Telescope Facility (SIRTF), Kepler, Eddington and Terrestrial Planet Finder (TPF) missions. Giant planets orbiting nearby stars can be detected using current technology, and the masses and orbits of these objects can be calculated. By combining these observations with simulations by our group, it will be possible to determine promising candidate stars for future missions and observing programs designed to detect Earth analogues, such as Kepler and TPF. We address the effects of methane on the Martian climate evolution. Our work concerning CO₂ clouds and the effects of impacts is relevant for interpreting data from Mars Odyssey and future

Mars missions, such as the 2009 Mars Science Laboratory rover. Our team's studies of cosmic ices and organics are synergistic with the Stratospheric Observatory for Infrared Astronomy (SOFIA), SIRTf and Stardust missions. The work with microbial ecosystems strengthens the systematics for interpreting the microbial fossil record and thereby enhances astrobiological studies of Martian samples. A team member serves as interdisciplinary scientist for astrobiology on the 2003 Mars Exploration Rover (MER) Mission. Models of biogenic gas emissions will enhance models of atmospheres that might be detected on inhabited extrasolar planets. One of us is first author on a major white paper that addresses the feasibility of searching for biosignatures during the proposed TPF mission. This white paper was just published in the peer-reviewed literature.

We have served the needs and interests of the nation's educators, students and public through a high-impact education and public outreach program. Specifically, we partner with the California Academy of Sciences (CAS), Yellowstone National Park (YNP), and the New York Hall of Science to develop new astrobiology workshops, activities, exhibits, and other products for the public. CAS has chosen to utilize astrobiology to link its natural history museum, planetarium, and aquarium under the theme, "Earth and its Place in the Universe." Ames personnel serve directly on the CAS design and exhibit development teams. Ames and CAS also facilitate interactions between researchers and educators in order to develop inquiry-based programs and activities for K-14 students. Our partnership with YNP combines a large annual visitation with a highly effective venue for conveying astrobiology-related content. With material input from the Ames team, YNP is introducing astrobiology content into trailside interpretive signs, brochures, and the Yellowstone Resources and Issues Guide. Ultimately, astrobiology will be integrated into permanent exhibits for the major visitor centers.



Figure 5. *The Trailside Sign Project represents the E/PO collaboration between the NAI Ames Team and Yellowstone staff, to allow park visitors to learn about Astrobiology by experiencing Yellowstone's natural features.*

We have extended this impact to the professional level by engaging graduate students and postdoctoral associates in the proposed research activities, through the teaching of undergraduate courses in astrobiology at Stanford University and at local community colleges.

Executive Summary: Arizona State University

An Overview of Astrobiology at Arizona State:

During our five years as an NAI charter member, Arizona State University sponsored a broadly based program of research and training in Astrobiology to address the origin, evolution and distribution of life in the Solar System. The following paragraphs provide an overview of the specific research areas pursued by the Arizona State University (ASU) Astrobiology team during Year 5. With such a large and diverse team, it is not possible in the space available to cover all details of the research progress made during the past year. For a more complete review, the reader is referred to the individual detailed reports for ASU that accompany this Executive Summary.

Section I: Origins of the Basic Building Blocks of Life

At ASU, the origins of living systems has focused primarily on understanding 1) the exogenous origins of carbon compounds through studies of the cosmochemistry of carbonaceous meteorites and 2) the endogenous abiotic synthesis of organics in deep sea hydrothermal vent environments.

A. Meteorite Cosmochemistry.

During Year 5, Co-I Laurie Leshin and her group continued studies of the chemistry of carbonaceous meteorites. By coupling accretion modeling of the early Solar System with D/H estimates obtained from meteorites, the group produced consistent water delivery scenarios for both the Earth and Mars (Lunine et al., 2003). In addition, Astrobiology Postdoctoral fellow Michele Minitti showed that the high D/H values of Martian rocks probably did not result from shock (Minitti et al. in preparation).

Research into nature of hydrous alteration environments on carbonaceous meteorite parent bodies was published by NAI Astrobiology Postdoc Gretchen Benedix in *Geochimica et Cosmochimica Acta* (Benedix et al. 2003). Using a combination of oxygen isotopic, electron microprobe and petrographic methods, this study established the nature and timing of aqueous alteration processes on the meteorite parent body. In addition, C isotope data provided new constraints on magmatic and surficial carbon reservoirs in the Martian carbon cycle (Goreva et al., 2003, Niles et al., in prep.)

B. Abiotic Synthesis of Organics in Hydrothermal Environments

Co-I's John Holloway and Peggy O'Day lead an effort to understand the endogenous (terrestrially based) synthesis of prebiotic organic compounds at high temperatures, specifically within seafloor black smoker hydrothermal systems. During Year 5, the group advanced a corollary to their previously published hypothesis that the degassing of seafloor basaltic magmas can provide hydrogen and carbon dioxide for abiotic organic syntheses (Holloway & O'Day, 2000), showing that the same processes can operate in conjunction with silicic magmas (Holloway, submitted). The team also continued to explore the synthesis of organic compounds from hydrogen and carbon dioxide in the presence of mineral catalysts under seafloor hydrothermal conditions. In this work they showed that smectite clay minerals, common in seafloor hydrothermal deposits, provided reactive sites for synthesis of complex organic compounds such as hexamethylbenzene and long-chain methyl esters from aqueous methanol solutions (Williams, et al., 2002; Canfield, et al., 2003).

The Holloway-O'Day group also applied synchrotron computed microtomography to characterize hydrothermal microhabitats by imaging the physical structure of young hydrothermal chimneys from 9°N East Pacific Rise. This analysis showed that the internal structure of sulfide chimneys consisted of a loosely connected network of euhedral mineral grains (predominantly sulfide by bulk X-ray diffraction analysis) and is dominated by diffuse flow (i.e., unchannelized). Imaging established that the physical structure of sulfide chimneys can indeed serve as an effective molecular sieve for the adsorption of organic compounds and bacteria from vent fluids and seawater. This provided the basis for formulating a model to show how organic compounds can accumulate on catalytic mineral surfaces under pre-biotic conditions.

Section II: Early Biosphere Evolution

The early evolutionary history of living systems on Earth has been addressed in a variety of ways at ASU, including ecological and molecular studies aimed at understanding the origins of photosynthesis, studies of microbial fossilization in modern terrestrial environments, with applications in interpreting the biosignatures preserved in ancient terrestrial (and extraterrestrial) geological materials, by novel approaches for reconstructing the paleoenvironmental conditions on the Archean Earth and early Mars and by understanding the role played by impacts in shaping the early biosphere.

The appearance of oxygenic photosynthesis is regarded as one of the pivotal evolutionary events in the development of Earth's biosphere in that the accumulation of photosynthetic oxygen at the Earth's surface and in the atmosphere ultimately paved the way for the origin of advanced multicellular life forms and human intelligence. Continuing controversy over the paleoenvironments and biogenicity of the oldest known terrestrial fossils, as well as the origin of putative fossil biosignatures in Martian meteorite ALH 84001, have emphasized the need for developing more robust chemical and morphological criteria for the recognition of ancient environments and fossil biosignatures in ancient rocks. At ASU, an interdisciplinary effort has been underway to address important aspects of the nature of Precambrian paleoenvironments and to define new approaches to fossil biosignature capture, preservation and detection in ancient terrestrial and extraterrestrial materials.

A. Origins of Oxygenic Photosynthesis:

During Year 5, Co-I Robert Blankenship led a group to study the origin and evolution of photosynthetic systems. This effort involved a broadly based interdisciplinary collaboration between a half dozen partnering universities. Over the past year, the Blankenship team continued their search for novel phototrophs by exploring the non-solar environments around hydrothermal vents. Deep sea hydrothermal vents emit a small amount of fluorescent light from both thermal and non-thermal sources. This emitted light has been suggested as a potential energy source for photoautotrophic organisms. Organisms collected during a 2001 field expedition to hydrothermal vents of the Nine North vent system of the East Pacific Rise are now in laboratory culture and being evaluated for their phylogenetic affinities and metabolic capabilities. Additional light measurements taken at vents along the Mid-Atlantic Ridge gave results similar to vents in the Pacific (White et al. 2003). This work on alternative energy sources holds important implications for the exploration for habitable environments elsewhere in the Solar System (e.g. deep ocean floor environments of Europa).

Field and laboratory studies of photosynthetic organisms in iron-rich environments showed that some photosynthetic organisms utilize reduced iron as an electron donor. This has important implications for understanding the origin of banded iron formations in the Precambrian and for interpreting the rise of atmospheric oxygen. The Blankenship team isolated and analyzed mutant proteins that are potential candidates for Fe-oxidizing complexes.

B. Origins and Preservation of Fossil Microbial Biosignatures

1. Microbial Fossilization Processes in Alkaline Lakes and Hot Springs:

The interpretation of biosignatures in ancient terrestrial and extraterrestrial materials depends critically upon an understanding of the processes of microbial fossilization, inherent biases in biosignature capture and preservation and the effects of diagenesis (post-burial alteration) on biosignature retention. The most relevant systems for study are those extreme environments where active mineralization processes operate and where environmental conditions provide good analogs for the ancient Earth, or other planetary settings. During Year 5 of NAI membership, a group led by Co-I Jack Farmer completed a study of the fossilization processes associated with oncoids (spherical stromatolites) found in the Rio Mesquites, Cuatro Ciénegas Basin, Central Mexico. Results were reported at the NAI general meeting and a manuscript is in preparation. This work was done in collaboration with James Elser's group, who have been studying the ecological aspects of the same system (see below). The work established that surface biofilms have a bi-layered community structure, with the upper zone being dominated by several groups of larger, mostly erect-growing filamentous cyanobacteria and many species of diatoms. Microelectrode studies showed that these surface species effectively control precipitation of calcium carbonate through photosynthesis. However, most mineralization is occurring at a depth of 1-2 mm below the biofilm surface in association with a subsurface community that is dominated by smaller filamentous species. Pervasive carbonate precipitation at this depth entombs the deeper community, preserving numerous cellular remains. Interestingly, species of the surface community are rarely preserved, creating a strong taphonomic bias toward preservation of the subsurface community.

Farmer's group also began a study to characterize biofilms and microbial fossil assemblages associated with low temperature carbonate-precipitating springs on the floor of Mono Lake, an alkaline-saline lake located in eastern California. (This comprises the topic of a PhD dissertation by Mike Thomas). The goal of the study is to trace the fate of biosignatures during carbonate precipitation, through early diagenesis. The results of the work in Mono Lake will be compared with ancient carbonate spring deposits elsewhere in the Mono Basin that were formed during the last glacial period when the lake was much deeper and colder. The work in the Mono Basin involves a collaboration with the Woods Hole team, who are focusing on the eukaryotic biodiversity of the lake biota.

2. Modes of Preservation in Precambrian Cherts:

Evaluating the nature of microbial biosignature preservation at the cellular scale is an important line of inquiry that

may eventually lead to robust criteria for the recognition of biogenic signatures in ancient materials. During Year 5, Co-I Sharp (Dept. of Geological Sciences) continued to apply Analytical Transmission Electron Microscopy (ATEM) methods to characterize the nanometer scale microstructures and composition of kerogenous microbial biosignatures preserved in the 2.0 Ga Gunflint Iron Formation. Electron Energy-Loss spectroscopy (EELS) confirmed that microfossils consist of amorphous kerogen concentrated along grain boundaries of micro-quartz. Kerogen is amorphous, with little evidence for graphitization. In coccoidal microfossils, kerogen forms cell-wall like features around cores of granular microquartz, whereas in filamentous forms, the kerogen is disseminated along needle-shaped grain boundaries that separate submicron-sized fibers of chalcedonic quartz. Sharp's team are also applying similar methods to the study of putative biosignatures in the controversial 3.5 Ga Apex Chert from Western Australia. The goal is to characterize the composition, crystallinity and distribution of the kerogen to determine if this material is of biogenic or inorganic hydrothermal in origin. This work is ongoing and forms the basis for a PhD dissertation by Brad DeGregorio.

3. Origin of Magnetite in Martian Meteorite, ALH84001:

The hypothesis of a biogenic origin for magnetite grains preserved in carbonates of Martian meteorite ALH84001 is the most compelling line of evidence for the discovery of Martian life by McKay et al (1996). During Year 5, Co-I Peter Buseck and colleagues continued their work with electron tomography and holography to evaluate the biogenicity of putative magnetite biosignatures in Martian meteorite ALH845001 (Buseck et al. 2002). The ultimate goal of the research is to determine whether the characteristics of crystal size distributions (CSDs) and shape factor distributions (SFDs) of magnetite obtained from magnetotactic bacteria can be used as mineral biosignatures. The group found that CSDs of magnetite obtained from 16 uncultured strains of bacteria showed both similarities and differences among crystals from bacteria from distinct localities and environments. Using a numerical method, the group sorted magnetite crystal populations based on features of the SFD of all particles and found that the numerical methods are useful for identifying bacterial magnetite in rocks. Basic conclusions may be summarized as follows: 1) Using electron tomography, there is too much uncertainty in any TEM results to date to confirm a biogenic origin for the magnetite in meteorite ALH84001; 2) Magnetite crystals in bacterial strain MV-1, the standard for comparison for ALH84001 magnetite, show variations within a chain and rounding of faces, which are not as well developed, or as crisp as in published interpretations; and 3) Progress has been made in developing automated procedures for electron tomographic data acquisition, reconstruction, and visualization.

C. Inferring Paleoenvironmental Conditions on the Early Earth (and Mars):

The initial colonization of the land surface marked an important event in the evolution of the biosphere. This opened a whole new dimension for biological habitation and dramatically expanded microbial involvement in global biogeochemical cycles.

During Year 5, Co-I Paul Knauth (Dept. of Geological Sciences) submitted a manuscript that describes the geologic context of the world's oldest land-based microbial fossil assemblage, dated at 1.2 Ga (Apache Group paleokarst, north-central Arizona). A second manuscript is in preparation that describes cave deposits associated with the Apache Group paleokarst and the putative microfossils entombed within these deposits. In addition, a paleontological study of modern caliche developed by the surface weathering of basaltic lava flows is also nearing completion. This particular study also demonstrates the potential of caliche as a host medium for capturing and preserving microfossils on Mars. (Caliche is a predicted weathering product on Mars, provided the planet once had a warm, wet climate).

E. Role of Impacts in Early Biosphere Evolution:

Asteroid and cometary impacts have been identified as an important environmental and evolutionary agent throughout the history of Earth's biosphere.

During Year 5, Co-I David Kring's group determined the distribution of impact-generated wildfires for the Chicxulub (end Cretaceous) impact event (~65 Ma) and illustrated how impact parameters (e.g., trajectory) can influence the distribution of wildfires (Kring and Durda, 2002). An invited review paper outlined the environmental and biologic effects of large impact events, like Chicxulub, throughout Earth history (Kring, 2003). The Kring group also continued their analysis of lunar impact melts, to determine the flux of impact cratering events in the Earth-Moon system, particularly during the first billion years of Earth's history (Daubar et al. 2002; Cohen et al. submitted). In an invited review paper, the group also explored the impact delivery of Earth's water, a key ingredient for life's origin (Campins et al., 2003) and initiated new directions in the study of impact-generated hydrothermal systems, habitats that may have been a particularly widespread and important during the early evolution of life on Earth.

F. Microbially-based Ecosystems, Cuatro Ciénegas Mexico: Windows for Lower Cambrian Ecosystem Structure

and Function.

The sudden appearance of complex, well-skeletonized invertebrates at the base of the Cambrian marks a singular event in the history of our biosphere. In an incredibly short interval of geologic time (<10 million years), representatives of all of the modern animal phyla appeared. With the addition of large herbivores and predators, a new global ecology emerged, replacing the microbial mat-dominated benthic ecosystems that had previously been the norm. The rise of large, multicelled bottom dwellers is broadly correlated with the decline of stromatolites, the fossilized biosedimentary structures produced by microbial mats. The disappearance of stromatolites has been attributed to competitive exclusion by algae and/or disturbance by invertebrate grazers. Whatever the cause, the nature of simple microbe-based/grazer ecosystems, the role of environmental and genomic factors in the evolution of such ecosystems and the nature of nutrients and energy flows provide an important context for evaluating the ecological and evolutionary context of the Cambrian explosion.

To better understand the ecological interactions that may have prevailed during the Cambrian transition, an interdisciplinary team of ASU and Mexican scientists, led by Co-I James Elser (Dept. of Biology) has been studying the ecology of simple, microbially based (fish-snail-microbial mat) ecosystems found in modern desert spring environments in the Cuatro Ciénegas Basin, Central Mexico. Goals of the study include an improved understanding the energy flow within such simple ecosystems and an improved understanding of the ecological factors that contribute to explosive evolution within highly endemic clades, the nature of ecological interactions between grazers and stromatolite-producing microbial mat communities and mechanisms of microbial fossilization. Ultimately this highly interdisciplinary study aims to test specific hypotheses about the ecological mechanisms that contributed to the Proterozoic decline of stromatolite-producing microbial ecosystems, as well as the ecological and evolutionary factors (particularly stoichiometric constraints on evolution, arising from disparities in C:N:P) that could have contributed to the Cambrian explosion that followed.

During the last year the team tested the hypothesis that the metazoan grazers of stromatolitic microbes face a stoichiometric constraint that results from consuming microbial biomass with elevated C:P ratio. This would lead to microbes having a high biomass C:P ratio due to extreme limitation of their growth by PO₄ in the well-illuminated, but oligotrophic spring fed environments that typify the study area. Sampling this past year confirmed that environmental levels of PO₄ at CC are indeed extremely low (<3 µg/L usually; levels of inorganic N are high) while organic matter in the surficial layers of various mats and oncolite stromatolites exhibits an extremely high C:P ratio, greatly exceeding values seen for other autotrophic communities in aquatic ecosystems and resembling the extremely nutrient-poor autotroph biomass observed in terrestrial ecosystems. The group also determined that PO₄ added to CC waters containing algal mats or stromatolites is removed extremely rapidly and results in a significant lowering of biomass C:P in the mat. Does such P-enrichment of microbial biomass improve the performance of metazoan grazers, as we have hypothesized? The first experiment to answer this question was performed during summer 2001, when a 2-week P-enrichment treatment lowered mat biomass from >3000 (by atoms) to ~900. Snails (*Mexithauma* sp.) grazing on P-enriched mats had higher RNA:DNA ratios than those feeding on unenriched control stromatolites (generally RNA:DNA ratio is considered a good indicator of growth rate). The encouraging results from 2001 inspired a larger, longer-term (2-month) experiment performed during summer 2002 when we performed a factorial experiment in which both PO₄ and the presence / absence of snails were manipulated. Data are preliminary but indicate that PO₄ enrichment lowered microbial C:P from ~1000 to ~100. However, in contrast to the 2001 experiment, snails on P-enriched stromatolites had lower RNA:DNA ratios and experienced high rates of mortality and significantly lower rates of tissue and shell growth. Thus, P enrichment in 2002 appears to have "poisoned" the snails. This outcome was perplexing but is comprehensible in light of recent findings related to another herbivore known to be often P-limited, *Daphnia*, which grows slowly when food C:P ratios are reduced to unnaturally low levels. It was hypothesized that *Mexithauma* has evolved in the presence of consistently low P availability and experiences routine P-limitation in nature; however, when exposed to unnaturally P-rich food, it suffers a growth penalty. Thus, the simple food webs at CC may be poised on a stoichiometric "knife's edge" with regard to phosphorus. Could the same have held true for the simple microbially based ecosystems of the lower Cambrian?

The team also conducted a series of studies using microelectrode profiling and bulk incubations to quantify calcification rates of stromatolites. This appears to be the first time this type of experiment has been accomplished on modern stromatolites. Measured areal rates of calcification were very high (~150 mg CaCO₃ cm⁻² yr⁻¹), similar in magnitude to those measured in tropical coral reefs. However, rates of bioerosion by invertebrates in the same oncolites, measured by analyses of fecal pellet production, are only slightly lower (~106 mg CaCO₃ cm⁻² yr⁻¹). This indicates that the system is in a precarious balance between net carbonate accumulation and destruction by consumers. This delicate balance could explain why stromatolites are so rare in grazer-dominated ecosystems, while

also providing support for the grazer hypothesis as an explanation for the Proterozoic decline of stromatolites. The results of this work were submitted to Science and are presently in review (Garcia-Pichel et al. submitted).

Another goal of the Cuatro Ciénegas study is to characterize the genetic and morphological diversity of its understudied biota (esp. microbes, cyanobacteria, and snails) in order to advance our understanding of the evolutionary forces that have affected species, especially the desert pupfish and snail species which exemplify extraordinarily rapid diversification under environmental extremes of temperature and salinity.

With regard to microbial diversity, Garcia-Pichel (et al, 2002) described associations of cyanobacteria that exhibit exceptionally novel adaptations for buoyancy involving calcite as ballast. In addition, a new form of extremely rare fresh-water red alga is in the process of formal description. Collaborator Valeria Souza (UNAM) isolated nearly 3500 separate strains of eubacteria and archaea from various habitats (benign to extreme) at Cuatro Ciénegas and characterized the DNA for 350 strains using RFLP with sequencing of the 16S rDNA in selected cases. Sequenced strains from cultivable bacteria include Gram-positive taxa (*Bacillus* and *Staphylococcus*) and an ample suite of Gram-negative forms (*Pseudomonas*, *Aeromonas*, *Aquaspirillum*, *Vibrio* and *Halomonas* predominate, while clones of uncultivable DNA show marine Archaea and taxa previously isolated at hydrothermal vents). Preliminary analysis of TRFLP patterns of CC microbes shows a moderate alpha diversity with few species dominating the community (within a site) and a very large beta diversity (between sites) where each site has its own species.

The high levels of morphological diversity were also demonstrated for the hydrobiid snail *Mexipyrghus* by Co-Is Carol Tang and Peter Roopnarine. Morphometric analysis of samples of *Mexipyrghus* populations have revealed an extremely high level of morphological diversification and differentiation among even closely adjacent habitats. A goal of future work is to examine the ecogenomic basis of this diversification and to determine if the high degree of morphometric variation documented is controlled by dominantly environmental or genetic factors.

Section III. Exploring for Life in the Solar System.

The active involvement of ASU astrobiologists in NASA missions to Mars and Europa has provided ongoing opportunities for research and training in the exploration for life elsewhere in the Solar System. Data from the Mars Global Surveyor (MGS) and Odyssey instruments have continued to provide the ASU team with basic mineralogical information needed to explore the past distribution of water on Mars and for identifying high priority landing sites for future Mars missions for Astrobiology. Orbital data from the Galileo spacecraft has allowed critical testing of the hypothesis of liquid water environments on Europa, Ganymede and Callisto and provided a basis for the selection of high priority landing sites for Astrobiology. Involvement of ASU astrobiologists in various Mars Program missions and mission planning efforts during Year 5 have continued to strengthen the NAI's contribution to NASA's missions.

Co-I Philip Christensen is Principal Investigator for three instruments on either current or planned missions to Mars, including the Thermal Emission Spectrometer (TES) instrument (onboard the Mars Global Surveyor (MGS) orbiter), the THEMIS instrument (onboard the Odyssey orbiter) and two mini-TES instruments (to be launched with the Mars Exploration Rover (MER) mission in 2003). Co-I Greeley is a former Chair of the Mars Exploration Payload Assessment Group (MEPAG), the primary community-based science strategy group for the Mars Program. Co-I Farmer was MEPAG Chair during 2002-03, was acting Chair of MEPAG's Astrobiology Science Steering Group during 2002-03 and is the current Chair of the NAI Mars Focus Group. Greeley and Farmer were members of the Mars Exploration Review Team (MERT) and MAST (Mars Ad Hoc Science Team) the past year, (each is an external oversight committee for the Mars Exploration Program). Greeley and Farmer are also participating scientists on the Mars Exploration Rover Mission and Phil Christensen is PI for the mini-TES instrument on MER. Co-I Laurie Leshin was PI for a Mars Scout mission proposal (SCIM).

A. Astrobiological Exploration of Mars.

Mars Global Surveyor Thermal Emission Spectrometer (TES) data continued to provide new information about the role of aqueous processes in shaping the history of Mars. Although no large-scale carbonate deposits have yet been detected on Mars, spectral evidence was obtained for the presence of H₂O-bearing minerals within Martian dust. Based upon spectral details of the dust, it appears that zeolites are a possible candidate for the aqueous mineral component.

TES data were also used to refine the candidate landing sites for the 2003 MER mission. Along with Gusev Crater, the specular hematite deposit at Meridiani Planum, discovered with TES data in 2001, was designated as a primary landing site for one of two Mars Exploration Rovers launched in 2003. On Earth, coarse-grained (specular) hematite deposits only form in the presence of large amounts of water and most are hydrothermal in origin.

Newly acquired images from the Thermal Imaging System (THEMIS) on the Odyssey orbiter were used to characterize the candidate landing sites at Merdiani Planum and Gusev Crater in much greater detail. Many new geologic features of these sites were revealed with stunning clarity using THEMIS data, helping to further refine the geologic context of the MER landing sites.

Masters student Alice Baldrige developed detailed mineralogical ground truth for remotely sensed analog sites for Mars located in the Badwater Basin of Death Valley. MASTER (mid-infrared spectral) data were used to identify the locations of mineralogically pure, end-member pixels (carbonate, sulfate and silicates) within the basin. To establish ground truth, end member pixels were located on the ground and sampled for detailed laboratory analysis of mineralogy. Laboratory methods included X-ray Diffraction, electron microprobe, electron microscopy, thin section petrography and point counting, lab and ground-based spectral analysis (using TES analog spectrometers). To aid spectroscopic identifications, a mid-IR spectral library was developed for evaporate minerals and added to ASU's spectral data base for use by the TES and THEMIS project teams who are presently mapping Mars. These spectra will also be used by the MER project team. Results of this study were used to establish abundance thresholds (for natural mixtures in the Badwater Basin) necessary for the detection of discrete evaporite deposits (especially carbonates, sulfates and silicates, including zeolites). Results suggested that at the coarse spatial of the TES instrument (3 km/pixel), the detection of carbonates and sulfates is unlikely. However, at the enhanced spatial resolution of THEMIS (100 m/pixel), both carbonates and sulfates should be easily detected, provided they are present at abundances exceeding ~15%. This work was submitted to the Journal of Geophysical Research-Planets and is presently in review (Baldrige et al. in review). The next phase of this research will examine the spectral resolution thresholds for the same data sets.

A second Masters study was also completed during Year 5 that explored the margins of the North polar remnant ice cap of Mars to search for sites of possible magma-cryosphere (volcano-ice). This work comprised a Masters thesis by Meredith Payne who has now entered the PhD program at ASU. The study commenced with broad photogeological reconnaissance using Viking data to identify potential water-formed geomorphic features. This was followed by detailed studies at four sites, selected to cover a range of potential aqueous processes. Hypotheses posed for the origin of geomorphic features were tested using Mars Orbiter Laser Altimeter (MOLA) data and Geographic Information System (GIS) tools (e.g. Digital Elevation Models) and comparisons to terrestrial analogs. In the course of this work, it was discovered that MOLA data are sensitive to subglacial topography in areas that have been recently deglaciated, but are presently covered by snow. The ability to "see through the ice" broadens our access to polar geological history based on MOLA topography (Payne and Farmer, submitted, Icarus). In addition, the hypothesis of a pseudocrater origin for a small field of cinder cone-like features was tested using MOLA data. It was determined that they are more likely be of a subglacial origin, but still formed by a process involving liquid water (Payne and Farmer, submitted, JGR-Planets).

A highlight of Year 5 was the publication of a paper by Co-I Christensen in Science that provided an alternative hypothesis for the origin of the numerous seep sites identified previously by Malin and Edgett (2001) based on Mars Orbiter Camera (MOC) data (Christensen 2003). The Christensen paper was based on THEMIS visible imaging that suggests that the seeps could have formed beneath snow-pack that accumulated during a recent period of low obliquity, and not by outflows of subsurface water (e.g. hydrothermal brines) as previously suggested.

Reconnaissance studies of Astrobiology Martian landing sites for the Mars Exploration Rover mission (successfully launched in June), as well as detailed studies of the Gusev Crater landing site, provided another major focus for the project during Year 5. Gusev was selected as the site for the first MER landing, scheduled to occur in January 2004, while the hematite site was chosen for the second MER landing a few weeks later. Results of these landing site studies were presented at community workshops, to the Mars Exploration Payload Analysis Group (MEPAG) and to the MER project team.

In addition to planning for NASA missions, Ronald Greeley has also participated extensively in planning for the Mars Express mission, which was also successfully launched by the European Space Agency in 2003. Greeley is a Co-Investigator on the High Resolution Stereo Camera System (HRSC) imaging team and provided a list of high priority imaging targets for Astrobiology to the imaging team for use with that instrument. Similarly, Co-I Farmer provided a similar list of high priority Astrobiology targets to the CRISM instrument team (CRISM is a hyperspectral near-IR spectrometer that will be launched to Mars in 2005).

B. Astrobiological Studies of Europa.

Co-I Ron Greeley's group completed a study of the "mitten" feature on Europa, which represents the extrusion of

ice onto the surface from a subsurface source (Figueredo et al. 2002). As such, the mitten structure comprises a high priority target for the future exploration of Europa to search for past or present life. Pole-to-pole geological mapping of Europa was also completed for strips representing the leading and the trailing hemispheres of Europa. This mapping was to explore for potential latitudinal or hemispheric asymmetries in ice fracture patterns. Studies of ice deformation in another region of Europa provided evidence for crustal fore-shortening, important for understanding deformation processes in Europa's crust. Each of the above activities helped to further characterize the nature and evolution of surface-near-surface environments on Europa needed to further assess the potential for habitable zones of subsurface liquid water. It was concluded from an analysis of domes and other features on Europa that they are geologically young and appear to have brought material to the surface from beneath the ice crust. Additional global geological mapping of Europa was also initiated during Year 5 as a first step toward identifying key sites for future surface exploration of Europa. Current data for Europa obtained by the Galileo project are also being analyzed to further understand the potential for european environments conducive for life (Figueredo et al. in press).

Executive Summary: Carnegie Institution of Washington

The team led by the Carnegie Institution of Washington is studying the physical, chemical, and biological evolution of hydrothermal systems, including vent complexes associated with ocean ridges, deep aquifers, and other subsurface aqueous environments, both on Earth and on other Solar System and extrasolar bodies. Such diverse systems are important environments for life on Earth and possibly elsewhere in the cosmos.

The traditional view of life's origin on Earth has focused on processes near the photic zone at the ocean-atmosphere interface, where ionizing radiation provides energy for prebiotic organic synthesis. In the context of astrobiology, this origin paradigm restricts the initial "habitable zone" around stars to planets and moons with surface water. According to this view, subsequent adaptations on Earth, and possibly elsewhere, led to expansion of the biosphere into subsurface habitats.

An alternative hypothesis is that life-forming processes may also occur in subsurface hydrothermal environments at the water-mineral interface. This hypothesis, that life on Earth originated from oxidation-reduction reactions in deep hydrothermal zones, perhaps at or near ocean ridge systems (Figure 1), opens exciting possibilities for astrobiological research. If a subsurface, high-pressure origin of life is possible, then the initial habitable zone around stars is greatly expanded to aqueous environments where redox reactions can be driven along thermal and chemical gradients.

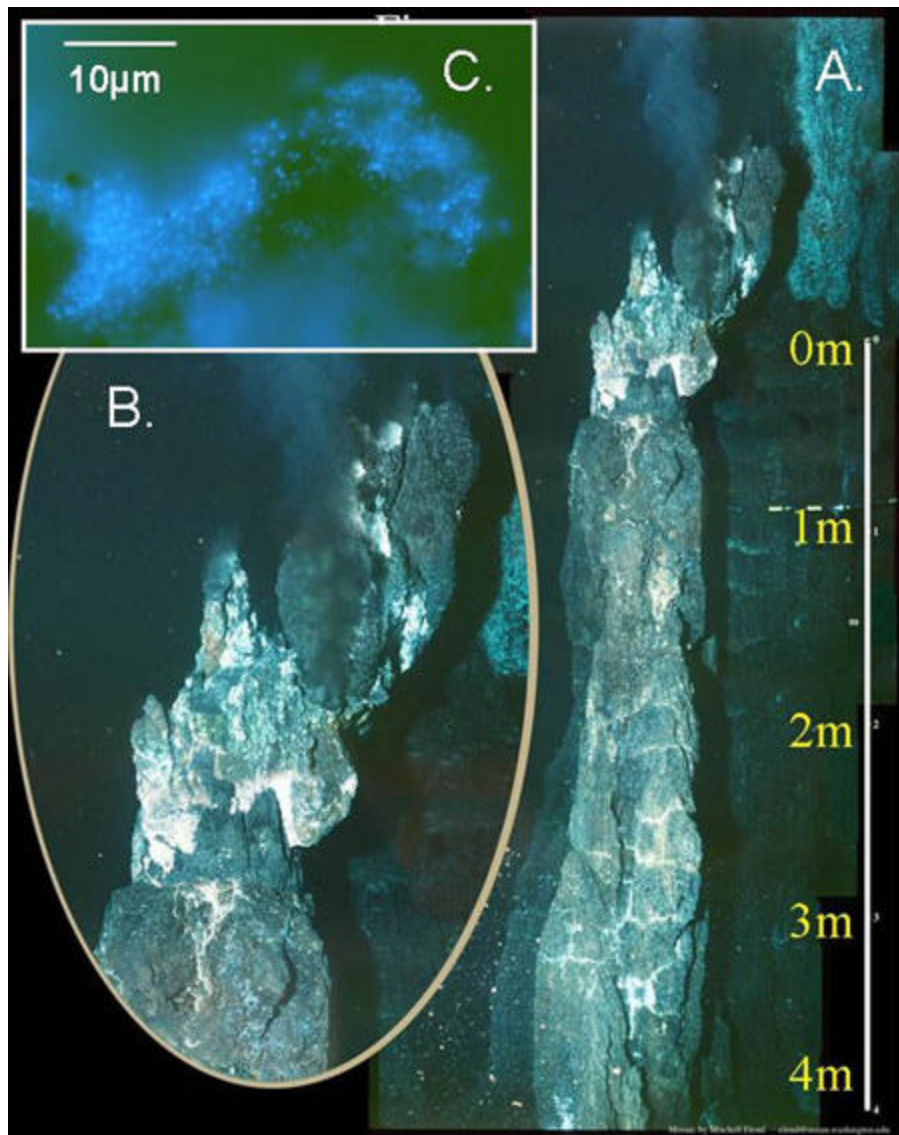


Figure 1. The black smoker sulfide chimney Finn (A) was recovered from the Mothra Vent Field on the Endeavour

Segment of the Juan de Fuca Ridge in July 1998. Finn was venting 302°C fluid upon recovery (B, close-up of Finn) and contained complex mineralogical gradients within its walls. The structure was sampled by Co-I Baross for co-registered microbiological and petrological studies. Attached microbial communities, some of which formed 10 µm-thick biofilms (C), were observed, by use of fluorescent probes, throughout the structure, including high-temperature regions near the central vent conduit.

Several lines of evidence lend credibility to the hydrothermal origins hypothesis. Numerous recent discoveries of high-pressure life, especially lithotrophic prokaryotes, suggest that hydrothermal environments support abundant life. Models of the Earth's formation postulate large, surface-sterilizing impacts as recently as 3.8 billion years ago, but deep hydrothermal zones may have insulated life from these traumas. Studies of molecular phylogeny reveal that thermophilic microbes are perhaps the closest living relatives of the last universal common ancestor. Finally, hydrothermal organic synthesis experiments reveal unexpectedly facile synthetic pathways. Whether or not life originated in a subsurface hydrothermal zone, these lines of evidence, coupled with the assumed widespread distribution of such environments in our Solar System and elsewhere, point to the need and opportunity for an intense study of the characteristics of hydrothermal systems.

Our consortium's research activities explore the physical, chemical, and biological evolution of hydrothermal systems from these complementary fronts:

- ✍ We model planetary formation, and we detect and characterize extrasolar planets, in an effort to understand the range of objects that develop hydrothermal systems as well as the distribution of volatiles, especially water, within those objects.
- ✍ We investigate the circumstances under which hydrothermal systems form on planets and other bodies and the expected physical and chemical characteristics of those systems as they evolve.
- ✍ We study geochemical processes in hydrothermal systems, especially those that lead to abiotic organic synthesis. A particular focus is the role of mineral catalysis in these systems.
- ✍ We consider the origin and evolution of biological entities in hydrothermal systems through studies of the biochemistry of contemporary hydrothermal organisms.

A complete understanding of hydrothermal systems and their role in life's origins requires substantial advances on all of these fronts, as well as an extensive and challenging integration of these topics. During the past year we achieved significant progress in each of these research areas, as well as increased attention to the interfaces among these theoretical, experimental, and field approaches.

Among the highlights from the past year's research in the area of **planetary formation and evolution** were the following:

- ✍ Ten new planets were discovered by high-precision radial velocity surveys, including the closest known analogue to our Solar System.
- ✍ A transit-planet search yielded three promising candidates for new extrasolar planets.
- ✍ Orbital stability of Earth-like, habitable planets in known extrasolar planetary systems has been constrained by theoretical modeling.
- ✍ Observations of suspected protoplanetary disks imply that planet formation must occur faster than previously thought.
- ✍ A new scenario for Solar System formation raises the estimated frequency of habitable planetary systems by a factor of five.
- ✍ A new hypothesis to account for the pattern of crustal magnetic anomalies on Mars suggests that these features may provide a basis for assessing early hydrothermal activity and hence possibilities for early life on Mars.

Highlights in the area of the **evolution of organic matter and water in meteorites** included the following:

- ✍ Chondrule formation may produce observable chemical signatures in a disk.
- ✍ CR chondrites contain the most primitive organic matter yet studied from meteorites. The range of compositions seen in chondrites result from aqueous alteration and/or thermal metamorphism under a variety of conditions on the different chondrite parent bodies.

- ✍ Pyrolysis of organic matter in interplanetary dust particles during atmospheric entry may have had an important influence on atmospheric chemistry early in Earth's history.
- ✍ Ion imaging has revealed for the first time clear evidence for a D-rich, but C-poor, component in interplanetary dust particles. This material most likely consists of hydrated minerals such as phyllosilicates and has a D/H ratio similar to that observed in cometary water.
- ✍ There is a hint that Martian meteorites preserve a record of the evolution of the Martian atmosphere, but the potential influences of shock and/or terrestrial contamination in the meteorites need to be better understood.
- ✍ Measurements have revealed isotopically heavy Fe ($\delta^{56}\text{Fe}$ ranging up to +0.5‰) in Fe-rich sediments associated with Fe-metabolizing bacterial colonies around hydrothermal vents on the Loihi seamount. This result holds out the promise that Fe isotopes will be a useful biomarker.

Highlights in the area of **experimental tests of proposed hydrothermal organic synthesis reactions** included the following:

- ✍ A robust abiotic chemical pathway from citrate to aspartate has been demonstrated. This pathway requires that citrate undergoes a retro-aldol reaction to form oxalacetate and that the oxalacetate has sufficient (albeit low) stability to remain long enough to be "trapped" as aspartate. Aspartate lies on the path toward pyrimidine synthesis and hence is a threshold into the ribonucleic acid (RNA) world.
- ✍ The facile conversion of nitrate and nitrite to ammonia at elevated pressures (to 0.2 GPa) and temperatures (to 250 °C) has been documented in the presence of 20 different transition-metal oxide and sulfide minerals.
- ✍ A surprisingly facile, straightforward course to chirality and functional polypeptides has been proposed. The pathway stems from the creation and survival of polyamino acids in impacting cometary bodies, a statistical demonstration of large fractions of chiral segments in the racemic polymers, and hydrolysis kinetics analyses showing the sole survival of the chiral segments.
- ✍ A reversal reaction has been demonstrated in the system $\text{FeO} + \text{CaCO}_3 + \text{water}$ by producing methane at 5 GPa pressure and temperatures greater than 1800 K. Such a result may have important implications for the generation of hydrocarbons in the hot, deep interiors of terrestrial planets.

In the area of supporting **theoretical studies of hydrothermal synthesis reactions**, a highlight was the following:

- ✍ Thermodynamic calculations support the possibility that sulfate reduction may be metabolically viable on Europa given the complex chemical processes thought to occur there.

Highlights in the area of **isotopic and molecular tracers of life** during the past year included the following:

- ✍ There is chemical evidence that the ancient (400-My-old), now extinct, vascular plant *Asteroxylon Milleri* had the biochemical capability for lignin synthesis whereas the slightly more primitive *Aglaophyton* did not. Identifying the point in the evolution of plants when lignin synthesis arose may provide insight into the vital evolutionary step that aided colonization of the continents by plants.
- ✍ The trophic interactions that characterized the ancient ecosystem preserved in the now-fossilized Enspel Formation in Germany have been reconstructed from the stable isotopic abundances of carbon and nitrogen.
- ✍ Preliminary data on five microbial species show mass-dependent fractionation of sulfur isotopes.
- ✍ Mass-independent sulfur isotope anomalies correlate with depositional environments of 2.5- to 2.7-Ga black shale and dolomite deposits from the Hamersley and Fortescue Groups, Western Australia.

Highlights in the area of **biological studies of hydrothermal systems** included the following:

- ✍ A characterization of microbes in subsurface fluids from Axial Volcano, Juan de Fuca Ridge, has demonstrated that there is a highly diverse community of bacteria and archaea that are unique to the subsurface of mid-ocean ridges.
- ✍ A community of potentially nitrogen-fixing archaea and bacteria that occupies the hot, anaerobic habitats of the mid-ocean ridge seafloor is not dependent on electron acceptors and nitrogen compounds produced by photosynthetic organisms.
- ✍ Studies of microorganisms within sulfide chimneys at active hydrothermal vents suggest that biofilm formation on minerals may be associated with microbial growth or survival at extremely high temperatures.

- ✍ The first phylogenetic characterization of a microbial community associated with a peridotite-hosted hydrothermal vent field has been completed.
- ✍ Deoxyribonucleic acid (DNA) microarrays for *E. coli* have been optimized to screen for changes in gene expression at high pressures.
- ✍ Isotopic analysis indicates that lithotrophic Fe-oxidizing bacteria appear to be heterotrophic, i.e., they obtain their energy from an inorganic energy source (Fe^{2+}) and their C from organic matter.

In the area of **new molecular recognition instruments for astrobiological applications**, highlights of the past year included the following:

- ✍ Antihopane antibodies have been developed for rapid screening of the presence of hopanes in extracts of soil samples.
- ✍ Optimization of organic and aqueous extraction protocols have been developed for the extraction of viable and fossil biomarkers for future use in robotic systems.
- ✍ Initial testing protocols have been designed to place these extraction techniques onto a lab-on-a-chip format.
- ✍ Microfluidic devices for the handling of sample extraction and inoculation of microarrays are in the design stage as part of a collaboration with Marshall Space Flight Center.

In summary, our team's recent research, including discoveries of new planetary systems, exploration of possible hydrothermal regimes on other worlds, elucidation of robust hydrothermal synthetic pathways, documentation of novel microbial metabolic strategies, and finding unexpected high-pressure environments for life, inform the central questions of astrobiology. Taken together, these discoveries are changing our views of life's origin and its distribution in the universe.

Introduction

The Harvard NAI team was constituted in 1998 as an interactive group of biogeochemists, paleontologists, sedimentary geologists, geochemists, and tectonic geologists assembled with the common goal of understanding the coevolution of life and environments in Earth history. The team originally proposed to focus multidisciplinary research on four critical intervals of planetary change: the early Archean (>3000 million years ago) when life began, the early Paleoproterozoic (2400-2200 Ma) when oxygen began to accumulate in the atmosphere and surface ocean, the terminal Proterozoic and Early Cambrian (750-525 Ma) when animal life radiated, and the Permo-Triassic boundary (251 Ma) when mass extinction removed some 90 percent of Earth's species diversity, permanently altering the course of evolution. Given reduced funding levels in years 1 and 2, however, the team chose to focus on the latter three intervals; over the past five years, team members have made substantial contributions to each research area. Fortunately, increased funding in years 3 to 5 and strong interest by additional colleagues at Harvard and MIT enabled us to expand both our membership and intellectual purview. Thus, to the three projects funded from the outset (and approached in fresh ways by our newest co-investigators), we have added four additional research foci. John Hayes' research at the interface of microbiology and biogeochemistry expanded as a result of incremental funding that enabled his group to undertake a substantial collaborative effort with the NAI Team at MBL; the addition of Dan Schrag (Harvard) in year 2 and Roger Summons (MIT) in year 4 further bolstered our team's collaborative research in biogeochemistry. In association with the Spanish Center for Astrobiology, Andrew Knoll completed research on Neogene iron formations in southern Spain that will play a direct role in guiding exploration of Mars hematites by the recently launched Mars Explorer Rovers (MERs). Charles Marshall pursued statistical analyses of molecular sequence and biostratigraphic data as part of the NAI Focus Group on Evogenomics. Lastly, Dan Schrag initiated collaborative research with MIT's Maria Zuber that addresses the enigma of Mars' carbonate-poor surface and, hence, the history of water on the red planet.

Scope of Team Activities in 2003-2004

Research by the Harvard team is interdisciplinary, attracting broad participation by scientists within the five member institutions (Harvard, MIT, WHOI, Rochester, Smithsonian Institution). We have also been successful in promoting cross-team collaborations -- research projects have been undertaken with colleagues from the Carnegie, MBL, Rhode Island, Penn State, NASA Ames, and JPL teams, as well as both the Spanish and Australian astrobiology centers. Moreover, our team participated actively in the Evogenomics Focus Group and took a leadership position in the Mission to Early Earth Focus Group (A. Anbar). Team members are active in research on novel biosignatures and digital mapping technologies that can be applied to solar system research; A. Knoll and J. Grotzinger are members of the 2003 Mars MER science team; and R. Summons serves on Mars Exploration Program Assessment Group (MEPAG) and the Astrobiology Science Strategy Group, committees charged with defining astrobiological research strategies for upcoming Mars missions. Equally important, research by Harvard team members on sedimentary and geochemical biosignatures as well as early states of Earth's atmosphere will directly influence plans for continuing planetary exploration and the projected Terrestrial Planet Finder mission.

During the past year, Harvard team members conducted field research in Australia, southern Africa, Svalbard, the Canadian Rocky Mountains, China, and Oman. We also taught actively at three universities and in the International Geobiology course, a summer course in geobiology for graduate students and postdoctoral fellows, sponsored by the Agouron Institute. In year 5, much of our Education and Public Outreach (EPO) effort focused on university teaching, but we also helped to develop a traveling museum exhibit on biology and Earth history, in partnership with the Harvard Museum of Natural History, and individual team members lectured to K-12 and adult groups.

Research Accomplishments in 2003-2004

Subproject 1: The Proterozoic Oxidation of the Earth's Surface

The history of oxygen in the oceans and atmospheres is thought to have played a key role in Earth's long term biological evolution. Ongoing research by Harvard team members addresses the initial oxygenation of the atmosphere and surface ocean 2400-2200 million years ago (Ma), renewed oxygen influx near the end of the Proterozoic Eon, and life and environments between those two events.

Research by H.D. Holland and colleagues has sharply constrained the timing of Earth's initial atmospheric oxygenation. Measurements of the isotopic composition of sulfur in pyrites from the Timeball Hill and Rooihogte formations, South Africa, show both a considerably larger range in $\delta^{34}\text{S}$ than older sulfides and the absence of a significant signal of mass independent fractionation of the sulfur isotopes. These observations provide strong evidence for the presence of oxygen in the atmosphere at the time these sediments were deposited. Re-Os dating of the pyrites yielded an age of $2,322 \pm 15$ Ma and an initial $^{187}\text{Os}/^{188}\text{Os}$ ratio of 0.1087 ± 0.0063 . The former value shows that the loss of a major $\delta^{33}\text{S}$ signal in the atmosphere occurred between 2,450 and $2,322 \pm 15$ Ma, indicating that O_2 appeared in the atmosphere between these dates. The latter number is essentially equal to that of the mantle at 2,322 Ma, indicating that the input of riverine Os to the oceans was minor, an inference consistent with the absence of Re enrichment in highly carbonaceous shales from this period. This, in turn, suggests that 2322 million years ago atmospheric oxygen levels were sufficiently high to eliminate the MIF signal in sulfur but sufficiently low to preclude significant oxidative weathering of Re and Os on the continents.

Research on mid-Proterozoic rocks by the Anbar and Knoll labs further contributes to our growing understanding of the redox development of the atmosphere and oceans. In 1998, Donald Canfield proposed that the cessation of iron formation deposition ca. 1850 Ma reflected the expansion of sulfidic deep oceans and not, as traditionally understood, the spread of oxygen throughout ocean basins. Research on the iron chemistry of the ca. 1500-1400 Ma Roper basin in northern Australia, completed by NRC postdoctoral fellow Yanan Shen, Knoll, and Australian Centre for Astrobiology colleague Malcolm Walter Research, demonstrated the presence of a strong redoxcline within this ancient marine basin. Indeed, Shen and colleagues have now shown that all northern Australian basins formed between 1730 and ca. 1400 Ma show evidence of basinal anoxia. The strong facies dependence of sulfur isotopic composition in Roper pyrites further supports the hypothesis that midway through recorded Earth history, marine sulfate levels remained well below their modern level. The Roper basin also contains abundant and exceptionally well preserved microfossils. Research in the Knoll lab showed that these fossils preserve complex wall ultrastructures, imaged by transmission electron microscopy (TEM), that document a moderate diversity of eukaryotic life in coastal environments of the mid-Proterozoic ocean. In complementary research, Ariel Anbar showed (1) that Mo isotopes can be used as paleotracers of redox conditions that integrate over the global ocean and (2) that Mo isotopic measurements of Roper samples support the hypothesis of relatively widespread anoxia in mid-Proterozoic oceans.

Subproject 2: Neoproterozoic-Cambrian environmental change and evolution

This subproject has enjoyed the broadest participation of Harvard team members, and for good reason. The Proterozoic-Cambrian transition witnessed remarkable changes in tectonics, climate, atmospheric composition, and especially life. This is the interval during which animal life -- and, hence, the prospect of intelligence -- radiated on Earth. Harvard team researchers are studying the paleontology (Knoll, Grotzinger, Erwin), geochronology (Bowring, Grotzinger), tectonics (Hoffman, Bowring), and environmental changes (Hoffman, Schrag, Bowring, Grotzinger) of this interval, with an eye to constructing models of integrated change in the Earth system.

Using new carbon isotope data from carbonate-rich Neoproterozoic and Early Cambrian sections in Svalbard, Namibia and Morocco, Paul Hoffman's group compiled the first high-resolution C-isotopic curve for Neoproterozoic-Cambrian seawater (Fig. 1). The curve highlights a series of high-amplitude biogeochemical anomalies that have no parallel in Phanerozoic (543 Ma to present) or earlier Proterozoic time. Two of these anomalies correspond to the Sturtian and Marinoan snowball Earth episodes, which were preceded uniquely by long intervals when fractional organic burial exceeded 0.4, or twice the modern value. Hoffman's group also found low-temperature equilibrium fractionation of carbon and oxygen isotopes between coexisting dolomite and calcite in the Marinoan cap carbonate sequence in Namibia and northwestern Canada. This suggests that dolomite formed in contact with seawater, unlike the normal Phanerozoic and Neoproterozoic ocean in which dolomite precipitation was kinetically inhibited. As sulfate ion is a known inhibitor of dolomite above 2mM concentration (versus 28 mM in modern seawater), Hoffman and students proposed that low sulfate concentrations, evolved in an ice-covered ocean, led to sea-floor dolomite formation during post-glacial transgression. Unlike normal marine carbonate phases, dolomite is stable and should be a more faithful carrier of isotopic information.

The dramatic diversification of animal phyla during early Cambrian time has fueled debate regarding the mechanisms of early animal evolution for over a century. What is now clear is that intrinsic catalysts, such as the innovation of developmental genetic mechanisms, as well as extrinsic processes involving environmental change, are both critically important in accounting for this major event in the history of life. Research by John Grotzinger and Sam Bowring on biostratigraphic, geochemical and geochronometric data from Oman lends strong support to the

hypothesis that environmental perturbation near the Proterozoic-Cambrian boundary facilitated the Cambrian radiation of bilaterian animal phyla. Biostratigraphic, carbon isotopic, and uranium-lead zircon geochronological data from Ara Group of Oman indicate an abrupt last appearance of latest Proterozoic calcified metazoans coincident with a large-magnitude, but short-lived negative C-isotopic excursion. U-Pb zircon age data from an intercalated ash bed directly constrain this negative excursion to be 542 Ma, consistent with previous constraints from Siberia and Namibia. The absence of calcified fossils in carbonate units above this negative excursion contrasts strongly with their great abundance in carbonate units below the negative excursion. Combined with the global biostratigraphic record, these new data strengthen hypotheses invoking mass extinction within terminal Proterozoic ecosystems at or near the Precambrian-Cambrian boundary.

Subproject 3: Permo-Triassic mass extinction and its consequences

At the end of the Permian Period, 251 million years ago, more than ninety percent of marine species disappeared; land ecosystems were similarly devastated. Harvard team members seek to understand the causes and evolutionary consequences of this greatest of all mass extinctions. During the past year, D. Erwin and S. Bowring have continued field and laboratory research on the timing of P-Tr mass extinction. During the same period, team member John Marshall and colleagues examined old models and developed new models of the oxygen cycle in the past and present oceans. In an earlier publication from this project, they demonstrated how it is difficult to achieve a widespread, sustained anoxia in models of the Late Permian Ocean. This has resulted in a dialog with a group at Penn State on the representation of the oxygen cycle in ocean models. Marshall and colleagues have used a model of the modern ocean circulation and biogeochemistry to explore the implications of the simplified parameterizations that are under scrutiny, finding that allowing negative oxygen concentrations is misleading, though it might be interpreted as "total oxidant," since it leads to large and erroneously identified regions of anoxia in modern ocean simulations. In addition to better parameterization, models of oxygen distribution in ancient oceans will require more detailed representations of oceanic nutrient and oxygen cycles. Marshall's team is developing such representations in the context of an atmosphere-ocean box model.

Subproject 4: Molecular and isotopic approaches to microbial ecology and Biogeochemistry

Ancient organic matter provides a rich source of biological and environmental information about the early Earth, as well as a potentially informative source of insights about Mars and other planets. John Hayes' group continued their investigations of the biogeochemistry of hydrogen isotopes, and this year completed meticulous analyses that demonstrate the quantitative nature of hydrogen isotopic "exchange" between organic matter and water. Hayes also completed field research on the Lost City hydrothermal system, using the research submarine Alvin to gather samples of lipid-rich fluids for biomarker and isotopic analysis. Team member Roger Summons continued his exploration of lipids in living microorganisms and ancient sedimentary rocks, characterizing the lipids of new isolates of extremophilic bacteria and archaeans and discovering molecular biomarker evidence for basinal anoxia in mid-Proterozoic basins from northern Australia (see above). Working together, Hayes, Summons, and MIT colleague Dan Rothman have proposed a novel explanation of the unusual record of C-isotopes in Neoproterozoic marine basins (see above). Their model suggests that high concentrations of dissolved organic matter in anoxic deep waters governed the isotopic composition of Neoproterozoic oceans.

Team member Dan Schrag focused on rates of methanogenesis in sediments from the South American continental margin. Vigorous upwelling off the west coast of South America leads to the deposition of organic rich sediments, creating chemical potential gradients that are host to diverse microbial communities. Ocean Drilling Program Leg 201 drilled several sites from this region last year. Degradation of organic matter generates extremely high alkalinity as well as large concentrations of dissolved ammonium and phosphate. High alkalinity drives the shallow precipitation of authigenic carbonate, resulting in depletions of dissolved Ca that mask that released by calcite dissolution (as indicated by increases with depth in dissolved Sr). Dolomite precipitation causes significant negative excursions of dissolved Mg from background down-core trends that are due to uptake during clay alteration. These excursions are coincident with sharply defined minima in Ca. Isotopic analysis of dissolved inorganic carbon carried out by our laboratory shows a pattern characteristic of sites dominated by methanogenesis. $\delta^{13}\text{C}$ values decrease sharply with depth in the first 10 to 50 meters, reaching values as low as -30 per mil, indicating organic matter oxidation as well as methanotrophy. Values increase below the zone where sulfate disappears, reaching a plateau at values between 0 and +15 per mil. In some cases, the sulfate content of the pore fluid increases again, because sulfate rich brine flows through the basaltic basement rock. Schrag is currently working with numerical models to calculate the rates of methanogenesis and methanotrophy to begin to ask questions about what limits the biological activity in these environments.

Subproject 5: Geobiology of Neogene hematitic sedimentary rocks

In 2003, NASA launched two rover missions to Mars. One of the rovers is slated to touch down in Meridini Planum, a region marked by aqueous hematite deposition. If we are to maximize the scientific opportunities of this mission, we must first complete careful studies of analogous systems on Earth, where biological and physical processes can be tied directly to paleobiological and geochemical patterns in deposited iron-rich sediments. The Rio Tinto drainage area of southern Spain offers just such an opportunity. During the past funding year, team member Andrew Knoll and colleagues from the Spanish Center for Astrobiology and the Johnson Space Center completed laboratory research on previously collected samples of present day Rio Tinto sediments and diagenetically stabilized iron rocks formed over the past two million years. The samples show how coarse grained hematites can arise during diagenesis of originally goethitic and jarositic sediments, and detail the macroscopic and microscopic textures that record biosignatures in hematitic rocks.

Subproject 6: Evogenomics (Collaborative focus group research)

Research by team member Charles Marshall focused on improving confidence intervals in paleontological estimates of evolutionary first and last appearances of taxa and on reconciling molecular clock and paleontological estimates of evolutionary divergence times. Research has also continued on the construction of a global database for the fossil record that will enable paleontologists to understand the history of biological diversity and facilitate the differentiation of the biological from geological signals in Phanerozoic marine diversity studies.

Subproject 7: Water and carbonates on the early surface of Mars

Although liquid water is not stable at the surface today, a large number of observations suggest that liquid water existed at least episodically at various times throughout Martian history. Although most morphological evidence for liquid water on Mars is consistent either with episodic and rapid release of water at the surface, or else with liquid water in the subsurface that results in chemical weathering reactions, the presence of valley networks and the degradation of impact craters on ancient surfaces of late Noachian age imply weathering and erosion by liquid water at the surface for substantial amounts of time. One particularly interesting aspect of Martian surface geology is the apparent discrepancy between the geological evidence for water on Mars during the Noachian and the lack of calcium carbonate on the surface. If a large body of liquid water on Mars persisted for millions of years or longer, the high CO₂ atmosphere would form carbonic acid, and react with the silicate crust, producing calcium carbonate. However, carbonate minerals have not yet been detected on the surface in sufficient quantities to be consistent with this hypothesis.

Team member Dan Schrag and MIT colleague Maria Zuber have begun to address this problem, hypothesizing that climate episodes warm enough to maintain an active hydrologic cycle endured only long enough to produce the erosional features, not long enough for calcium carbonate to reach saturation. They are currently modeling this scenario for different ocean volumes and different atmospheric CO₂ concentrations.

Executive Summary: Indiana University (Bloomington)

Our decision to seek membership in NASA's Astrobiology Institute is motivated by the desire to bring our expertise in deep subsurface ecosystems to bear on the scientific and technological difficulties that will be encountered during the exploration of life beneath the surface of Mars. Our center will be a consortium composed of 9 senior level investigators, three of which are from DOE laboratories (PNNL, LBNL and ORNL) and five from academic institutions (Univ. of Tennessee-UTenn, Indiana University-IU, Univ. of Toronto-UT and Princeton University-PU) and one from Lunar Planetary Institute. We hope to complement the already excellent research being performed by NAI on the Mars focus area and believe our center's expertise in subsurface ecosystems, and access to unique facilities and field sites will enable us to develop synergistic relationships with other biological, geological and planetary research in NAI.

Our NAI would focus upon subsurface microbial communities that have been sequestered from the surface photosphere for tens to hundreds of millions of years and the environments that support their in situ activities. These terran ecosystems would represent the closest analogy to what might exist beneath the cryosphere of Mars. We seek to characterize the microbial, mineralogical and geochemical interactions, the isotopic signatures of the organic and inorganic gaseous, aqueous and metallic species, the interspecies and interkingdom communications and interactions, the genomic diversity and capabilities, the proteins expressed and their origin and the metabolites created and exchanged. This information will be used to design life detection approaches that will be tested in well-characterized field locations as a first step towards the design of flight-capable life detection instruments for future Mars drilling missions.

The proposed research will address five of the seven goals outlined in the Astrobiology Roadmap (<http://astrobiology.arc.nasa.gov/roadmap/>). The study of deep subsurface, ecosystems in ancient groundwater is directly relevant to the exploration for extant life in the subsurface of Mars, Objective 2.1 of Goal 2-Life in Our Solar System. In geological terranes where thermal overprinting has eradicated microbial life and which have remained isolated from the surface since that thermal episode, our investigations will determine the type of prebiotic compounds that can be formed in the subsurface and whether life itself could have been spawned beneath the planet's surface. Hence our proposed research touches upon aspects of Goal 3-Origins of Life. Because we have accessed our deep subsurface ecosystems by way of the deep Au mines of South Africa, characterization of the geochemical, lipid and isotopic signatures preserved in these rocks will enable us to answer some of the questions raised in Goal 4-Earth's Early Biosphere and its Environment. We will explore the evolution, environment and limit of life, Goal 5, by examining the community composition of subsurface ecosystems in different geochemical venues and by performing in situ experiments to see how the community evolves in response to environmental changes. Our research is particularly relevant to Objective 5.3, the determination of survival strategies that permit organisms to maintain viability in a radioactive environment for millennia. Finally, our research is already identifying isotopic signatures that indicate the presence of subsurface life, Objective 7.1 of Goal 7-Signature of Life, and our research plans will determine whether these signatures are preserved in the rocks.

Education and Public Outreach (EPO) activities in IPTAI are designed around three areas of emphasis. First: educational workshops for undergraduates and high school teachers where participants actively collect and interpret data from laboratory and field experiments. Second: public outreach through a web site with premiere-quality digital media including animations and video that illustrate how and why scientists conduct research in deep mines. Third: mentoring undergraduate and graduate research at Indiana, Princeton, and Tennessee universities. These astrobiology students will work with faculty to design a series of web-based quantitative and investigative activities for all pre-college students but highlighting the diverse careers of leading women on the IPTAI team.

Inclusion of collaborators from the School of Fine Arts, IU Instructional Support Services, and University Information Technology Services at Indiana University is an unusual aspect of this proposal. High-resolution digital video/audio materials will be collected during field experiments and will be use in both research and educational components of the IPTAI. Videos produced by scientists will document research methods in a substantially different way from conventional commercial films. We hope to capture examples of both set-backs and advances in research resulting from unanticipated and challenging conditions in deep mines. Given severe time and access constraints in deep mines, digital documentation of the physical conditions and the configuration of instruments are essential for interpretation of experimental results.

The proposed IPTAI offers unusually high levels of institutional commitment from Indiana University, Princeton University, and the University of Tennessee. The total matching funds from these three institutions is in excess of \$1,000,000. The match includes technical staff, graduate student stipends and tuition, academic salary, renovated

space for an institutional office, and travel and supplies for E/PO. In addition, the level of technical support for data storage, videoconferencing, and production of premiere-quality digital media at Indiana University is among the highest in the nation.

Executive Summary: Jet Propulsion Laboratory (JPL)

The fifth year of the JPL/Caltech/USGS NAI group was a great success with regard to discovery, publication of seminal work, representation of the work to the public, and overall adherence to the roadmap objectives espoused by the NAI. The work was highlighted by several keynote publications in the areas of life detection and biosignature development and testing, and set the stage for future work in the area: work critical to the assessment and testing of instruments for life detection missions. Each Co-I trained several students and postdocs, presented papers at the NAI annual meeting, and many of them presented plenary lectures at national and international meetings. Some of these are highlighted below.

As has been stated previously, the goal of our program slowly evolved from that of the co-evolution of Earth and its life, to that of a goal focused more centrally on the definition of life, the development of methods for its detection, the testing of some of these methods, and the beginnings of moving these methods to mission readiness.

The Co-Is of the program in the last year included:

- ✍ G. Blake and Y. Yung, Caltech -- Isotopes of nitrogen oxides as biosignatures
- ✍ J. Kirschvink, Caltech -- Microbial magnetosomes as biosignatures
- ✍ T. Ahrens, Caltech -- The effect of impact on potential biosignatures
- ✍ G. McDonald, JPL -- Organic geochemical biosignatures
- ✍ M. Fogel and D. Rumble, CIW -- Stable isotopes of C and S as biosignatures
- ✍ C. Johnson, Univ. of Wisconsin -- Stable isotopes of Fe as biosignatures
- ✍ J. Banfield, UC Berkeley -- Mineral weathering as a biosignature
- ✍ A. Anbar, U. Rochester -- Stable isotopes of metals as biosignatures
- ✍ K. Nealson, JPL/USC -- Development of strategies for life detection

For each of these areas, significant progress was made, papers were published in major journals, as noted in the individual reports, and national and international presentations were made. Some of these are highlighted below:

Of particular note, and delineated in the individual progress reports, was the work of **Dr. J. Kirschvink**, who has been a proponent of microbial magnetosomes as biosignatures. His work on magnetosomes produced a number of published papers, generated great interest in this area, and continues to be interpreted as a potential biosignature both in subsurface samples on Earth, and in extraterrestrial samples, when returned. This work involved collaboration and interactions with members of the NAI from several centers, and within our own group.

In addition, **Dr. Clark Johnson** has continued his work with stable isotopes of iron, one of the areas that represents one of the seminal discoveries of our NAI program, and one that has attracted major attention in the international arena.

Dr. **Gene McDonald** of the JPL made considerable progress using amino acids as biosignatures to study several different earthly sites, and developed several approaches for applying these methods to extraterrestrial samples. In addition, in collaboration with members of the CIW group, analyzed tholin compounds with the goal of modeling their production and properties for the upcoming Cassini mission.

Members of our team have been involved with a number of NASA groups including the roadmap writing exercise, Mars Exploration Assessment Group (MEPAG), as well as with several ongoing missions, including Mars Global Surveyor, Odyssey (Nealson is a Co-I on Thermal Emission Imaging System (THEMIS)), and have participated in the writing of several new proposals for missions.

Finally, members of the JPL-1 team have been outstanding in terms of public interactions, presenting major talks at many meetings, both national and international. These include plenary lectures at several conferences, many lead talks at Gordon Conferences, and talks at international meetings. These talks have ranged from general talks about life detection strategy to very specific talks involving individual biosignatures being studied by each PI.

Executive Summary: Johnson Space Center

The JSC Astrobiology Center is very diverse team that has about as many non-JSC members as it does JSC members. Since we are unable to add new civil servants, only 3 civil servants participate significantly in any of the projects or the education and outreach activities, and only two of them can spend significant time on the science projects. Most of the JSC team consists of contractor scientists, two of which are also graduate students while working at JSC, summer student interns, a NAI post-doctoral fellow, and scientists from nearby institutions. Our team represents a variety of disciplines, including geology, mineralogy, microbial extremophiles, geochemistry, and planetology.

The uniting theme for the JSC team is sample and material analysis to provide characterization data on terrestrial samples, astromaterials, and experimental samples. The types of data sought are usually features related to microbial life and the byproduct of microbial activities. Such features include morphology of living microbes and their surroundings, including biofilms on geologic substrates, as well as the morphology of fossilized forms of this living biota. In addition to morphology, chemistry and mineralogy of microbial produced features and fossilized microbes are of great interest to us. We typically using probe instruments (scanning electron micrograph (SEM), transmission electron microscopy (TEM), electron microprobes, TOF-SIMs, double laser beam mass spectrometers, and Confocal and epi-fluorescence microscopy to acquire information on living or dead microbial life. The overall goal is to relate chemistry, mineralogy, atomic structure, morphology, spectral interactions, and other properties for specific types of features so that multiple data can be used to characterize or fingerprint life in all its forms (including ancient fossils and bioassisted mineral precipitates). But we also strive to understand the processes that create the characteristics of life, the processes that alter or fossilize life, and the processes that produce and preserve interaction between life forms and rocks or minerals. Fingerprints of life are valuable for themselves, but they may also contain the history of that life and its environment over time.

The ability to confidently identify the presence of life or the former presence of life has been taken for granted in many types of terrestrial samples. However, as we are looking more and more at astromaterials from beyond Earth and taking a fresh look at terrestrial Archean samples, many in the scientific community have realized that it is not always obvious or easy to determine whether life was or is present in the rocks, minerals, soils, and fluids of Earth or another planet.

Consequently, our overall goal is to develop better techniques for detecting and understanding life and develop a biosignature classification system that assigns biosignature potential ranked by probability. It will be absolutely necessary to do this for returned Mars samples, but these techniques should first be well tested on terrestrial samples, and should also be applied to other types of samples as well: meteorites, cosmic dust, cometary dust, and samples from various satellites and small bodies. Because of the extreme interest in the possibility of life elsewhere, we must develop strong criteria for detecting it and certifying it. That is the current overreaching goal of the JSC team. That may also become the core objective for all of NASA in the coming decade or two.

Projects

During the past year we have made progress on some ongoing projects and have started several new ones. A brief description of each project and a few highlights of our progress is given below:

Publications:

During the past 12 months, our JSC Astrobiology Institute team has published more than 100 scientific papers and abstracts:

Peer-reviewed publications: 73

Published abstracts: 87

Total 160

The peer-reviewed publications include book chapters. Most of the abstracts are extended abstracts. Most of the published abstracts were also presented at meetings as talks or sometimes posters. In addition to talks associated

with published abstracts, we gave at least 25 more scientific talks to various meetings and technical groups, amounting to about 90 scientific talks and posters total.

Science Projects

We have subdivided our several small projects into five main work projects listed below:

1. New Technique Development and Application

- ✍ Continued development of using antibodies and state-of-the-art array approach may be used to search for life on Mars on robotic missions.
- ✍ Hopanes, a reliable established class of chemical biosignature, has been successfully detected in ELISA tests during Mars microgravity experiments performed for NASA's KC-135 flight program.
- ✍ *Limulus Amebocyte Lysate (LAL) and Prophenoloxidase system (PPO)* -- We have developed very sensitive methods to detect microbial contamination based on the *Limulus Amebocyte Lysate (LAL)* and *Prophenoloxidase system (PPO)*. These methods are to detect LPS, glucan and peptidoglycan down to sub-picogram level in pharmaceutical applications that have adapted them to rock, soil and metallic surfaces.
- ✍ Use of multiple fluorescent probes gives a visual representation of disseminated organic material in geologic materials such as interplanetary dust particles (IDPs) and rock varnish.
- ✍ New analyses of Nakhla, Mars meteorite, by double laser spectrometry shows presence of complex organics.

2. Organic Biosignatures

- ✍ We have used antibodies to detect category 1 and other biomarkers in rock samples. Extraction takes a few minutes and analysis a few hours.
- ✍ We have presented use of new antibodies to detect hopanes and have shown proof of operation during Martian gravity.
- ✍ The analysis of the carbon in AHL84001 carbonate globules is organic carbon. The C-ring analyzed is consistent with the detection of polycyclic aromatic hydrocarbons (PAHs) associated with the carbonate globules. We found that carbon was approximately uniformly distributed throughout the carbonate, although the C-ring and C=O to total carbon (which is mainly in carbonate) is highest in the rim carbonate.

3. Terrestrial Analogs and Martian Meteorites

- ✍ We discovered novel strains related to the bacterial genera *Coprothermobacter*, *Geobacillus*, *Clostridium*, *Anaerobaculum*, and also a previously uncultivated spirochete in ultradeep gold mines in South Africa.
- ✍ Microbial communities in hypersaline environments suggest that mineralization is controlled through biofilm production, and extreme desiccation of those films contributes to evaporate mineral deposits on a micrometer scale.
- ✍ Atacama Desert bacteria isolates suggest variation of species through elevation changes.
- ✍ Mn carbonates with complex textures were found in Nakhla.
- ✍ Rock varnish preserves C disseminated throughout the varnish microlayers, but is generally a poor morphological preserve for bacteria in older varnish samples. Hematite in the banded iron formation has a better preservation of microorganisms than seen in older ferricrete or hematite in rock varnish.
- ✍ A variety of microbes and viruses thrive in permanently ice-covered Antarctic lakes, even though the ice filters out more than 98% of the light.
- ✍ "Hot" (2°C) springs emerging from the upper surface of some Canadian Arctic glaciers contain microbial life.
- ✍ Carbonate globules in igneous rocks are extremely complex and are often associated with secondary silica-rich phases -- preliminary evidence suggests that this silica phase forms at relatively low temperatures and is not a melt glass.

4. Mineral Biomarkers

- ✍ Tiny magnetite in ALH84001 was confirmed as indistinguishable from magnetite made by bacteria.
- ✍ Magnetite in ALH84001 continues to strongly support the early life on Mars hypothesis.
- ✍ Manganese minerals formed in caves and on rock varnish with the help of bacteria may provide possible biomarkers for use on Mars.

- ✍ Transmission Electron Microscopy tomography has confirmed the original geometry MV-1 and ALH84001.
- ✍ Nanobacteria may have ribonucleic acid (RNA) and nucleate calcium phosphate precipitation.
- ✍ Recent experiments show that Dolomite has demonstrated that sulfate reducing bacteria may initiate the formation of this mineral.

5. Archean Biosignatures

- ✍ Hydrothermal systems preserved in the Archean Pilbara succession suggest that abiotic hydrothermal processes dominate sedimentation, making it very difficult to isolate biotic signatures.
- ✍ In the search for signs of microbial mats in zones of quieter sedimentation away from vents, promising results have come from fine-grained clastic sediments in the Paddy Market Formation. The formation consists of thinly laminated shales and sandstone -- probably turbiditic in origin.

Education and Public Outreach

Our Education and Public Outreach (EPO) program is one of the most active in the NAI. Their work his past year includes workshops, classroom activity product development, display events, CD authoring, public presentations, and the support of many undergraduate interns. We helped design and build the major museum exhibit called Microbes!, which is continuing its tour of major U. S. science museums. Members of the JSC Astrobiology Institute Team held more than 50 press interviews, TV and radio appearances, and other media events during the past 12 months. In addition, we made dozens of general public presentations and classroom presentations around the world. All of these Education and Public Outreach projects are documented in the detailed report. We believe we have an exemplary program in this area and we intend to continue its rapid pace.

Collaboration

We have put together two teams from Historically Black and Hispanic Universities and have helped them get independent funding through proposals. We now have an Astrobiology group at Highlands University in New Mexico, which is a subunit of the JSC Astrobiology Institute. In addition, a member of our local team (Penny Morris-Smith) has been awarded a significant grant to incorporate minority students and from Texas Southern University, the University of Houston (downtown campus) into some of our projects as interns, visiting faculty, and graduate students. We will also work with the Houston Museum of Natural History on this project. In summary, we have added three universities to our active group, all specifically in astrobiology. We have raised more than half a million dollars from other sources to support these efforts.

We currently also have a Memorandum of Understanding in negotiation between NASA Headquarters to work with a group at the Geological Survey of Canada specifically on Astrobiology topics, generally focused on biosignatures. This MOU was signed this past calendar year.

As indicated in our science project reports, we are now actively working with a number of universities as documented also in previous progress reports. These collaborations have proven to be valuable and have provided our team with considerable expertise, which we originally lacked, specifically in microbiology, genomics, microbial ecology, and field and laboratory procedures for sampling, culturing, preserving, and working with microbes. However, we now have two new astrobiology labs at JSC to continue nanobacteria and Microarray Assay for Solar System Exploration (MASSE) hopane research, the MASSE extraction test bed prototype development and a lab for clean and sterile preparation of geologic samples and astromaterials. These new laboratories opened May 2003 to better serve our research collaboration with Carnegie, Montana State and the University of Arizona. We are now capable of interacting better with other NAI teams on a scale that we have not had before.

We also have an established a working relationship with the Madrid Center for Astrobiology for work on the MASSE project. They are helping develop the array technology and we are working on the antibody-antigen reactions. We will both participate in final design and in the test program, and they will start field tests at the Rio Tinto in September of this year. They will be partners on any flight hardware that we are successful in getting onto a mission.

In addition to the Centro de Astrobiologia (CAB), we also have an established and strong partnership with Carnegie Institute through MASSE PI, Andrew Steele. His project engineer and PostDoc are housed at JSC, utilizing our new laboratory space.

Overall, we believe we have made considerable progress this year and we look forward to more collaboration with other NAI members in the coming year.

Executive Summary: Marine Biological Laboratory

Environmental Genomes and the Evolution of Complex Systems in Simple Organisms

Biology imposes an overwhelming force on planetary change through biogeochemical processes that originated in ~3.5 billion year old microbial communities. Through metabolic activities, microorganisms orchestrate key processes in carbon fixation, geochemical cycling, biodegradation and atmospheric change. For at least 80 percent of our evolutionary history, microbes were the only forms of life. By comparison, the multicellular world of plants, animals and fungi are derived forms whose continued existence is completely dependent upon a microbial world of uncharted diversity. Whether biological systems similar to those on Earth ever occurred or continue to function on other planets or large satellites is unresolved but if life occurs elsewhere in our solar system, it will be microbial in form.

The primary objectives of the Astrobiology Team at the Marine Biological Laboratory are rooted in delineating the evolutionary history of microbial life, exploring the nature of early metabolic processes, and defining the limits of the "habitable zone". We seek to understand patterns and mechanisms of genome evolution and metabolic variation that allowed diverse microorganisms to adapt to new environments, to generate novel phenotypes, and to evolve processes that cause environmental changes on a global scale. Key questions include: When and where did life originate? How did biological complexity develop from emergent properties of living organisms? Which metabolic processes were significant drivers in modifying early Earth? What are the limits of extreme environments compatible with life? Answers to these questions will have a direct impact on how to organize and target exploration in search of living organisms beyond our own biosphere.

Our general strategy capitalizes on advanced molecular biological techniques and ranges from analyses of genomes to holistic studies of individual organisms within microbial communities. The physiological and microbial diversity studies focus on the hydrothermally altered sediments of Guaymas basin in the Gulf of California, the acidic, heavy metal laden Río Tinto of southwestern Spain, and photosynthetic cyanobacteria from microbial mats.

Evolution of Proteins: a genomic approach. The study of complete genome information from even a single organism can provide important insights about how metabolic diversity evolved from a relatively small number of catalytic centers that must have been present in early proteins. For example, fatty acid biosynthesis is an important pathway for every organism. It is a multi-step process and one of the key enzymes in the biosynthesis of lipids is fadB.

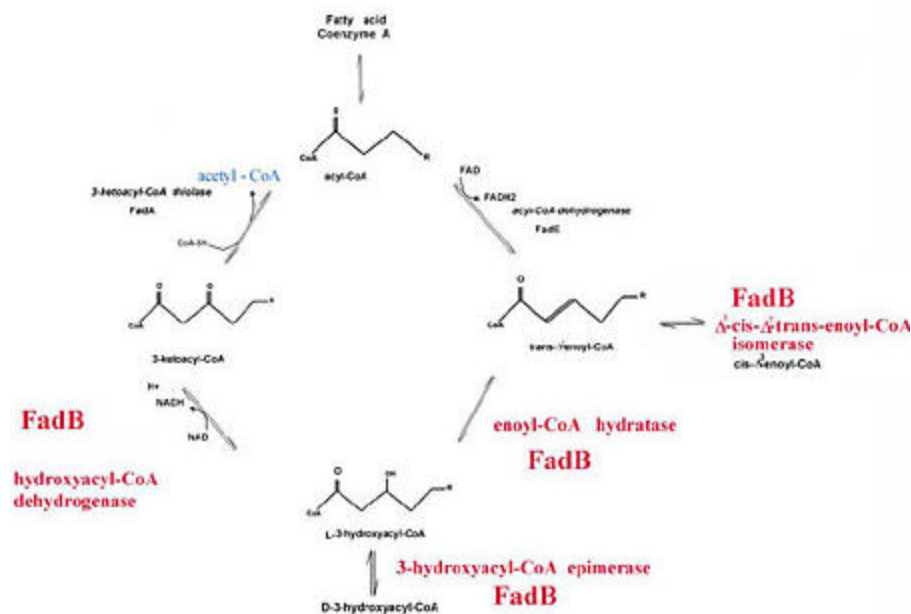


Figure 1. *The Pathway ([?]) oxidation of Fatty acids*

Using the N terminus as a probe for in silico studies, Dr. Riley and her colleagues mapped domains of the fadB sequence onto known structures of enzymes that catalyze reactions as diverse as epimerase, isomerase, hydratase

and protease. They demonstrated that the C terminal part of the *fadB* protein has a dehydrogenase activity, whereas a single catalytic site is structurally related to epimerase, isomerase and hydratase.

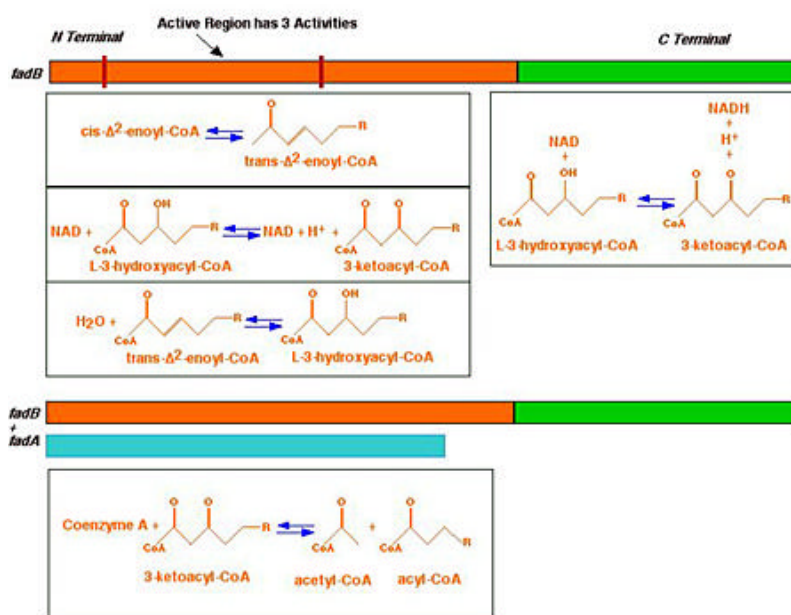


Figure 2. *The Reaction Mechanism*

The proteins of the hydratase/isomerase superfamily catalyze a very diverse range of reaction types using a structurally conserved active site fold. This reinforces the idea that a relatively small number of catalytic domains in early proteins gave rise to metabolic complexity found in contemporary cells. The basic signatures of these seminal domains could provide the basis for design of life detection strategies.

Diversity and Evolution of MicroOrganisms.

Woese's seminal work funded through NASA's original Exobiology program unveiled a Universal Tree of Life that included three primary lines of descent; the Archaea, Bacteria and Eukarya. The MBL Astrobiology team continues to use and develop new molecular methodologies that can more efficiently describe microbial evolution, microbial diversity and how these organisms function in extreme environments. This information will expand our definition of habitable conditions and may suggest new targets for developing life detection capabilities. We now seek greater resolution in studies of microbial population structures as well as information about their association with specific metabolic functions. For example, we have explored the phylogenetic and functional diversity of sulfate-reducing bacteria in the sediments of Guaymas Basin (Gulf of California). In this hydrothermal vent site, thermal alteration of deposited planktonic and terrestrial organic matter forms petroliferous material. These hydrocarbons are a significant carbon source to the vent microbial communities, which includes diverse sulfate reducing bacteria.

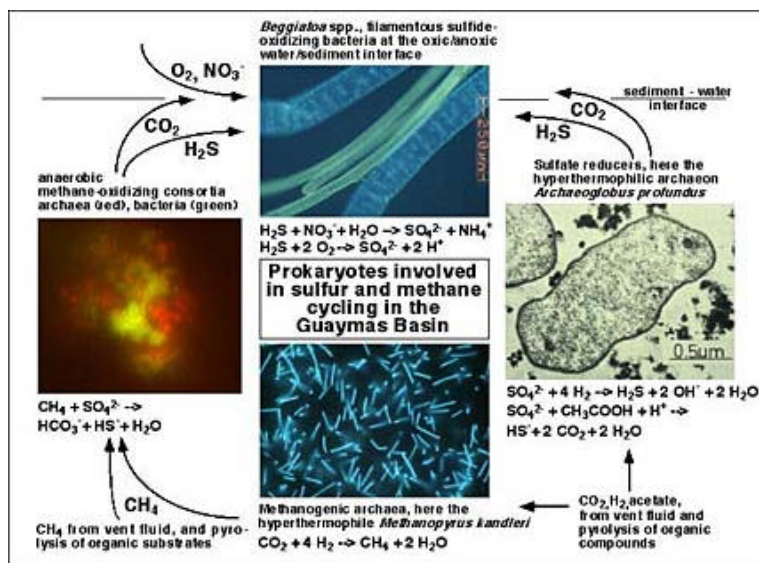


Figure 3. Microorganisms and their reactions (sulfate reduction, methanogenesis, methane oxidation, sulfide oxidation) in the methane and sulfur cycles in the Guaymas Basin hydrothermal vents.

Using conserved primers, we polymerase chain reaction (PCR) amplified dissimilatory sulfate reductase genes, the key gene for sulfate reduction (*dsrAB*), and 16S ribosomal ribonucleic acid (rRNA) genes from the upper 4 cm of Guaymas sediment. Figure 3 is a phylogenetic analysis of the nearly full length *dsr* sequences. In this analysis the *dsrAB* sequences revealed a major clade that branched with *dsr* sequences of the genus *Desulfobacter*, acetate oxidizers of the family *Desulfobacteriaceae* within the delta *Proteobacteria*, and a clade of novel, deeply branching *dsr* sequences related to environmental *dsr* sequences from marine sediments in Aarhus Bay and Kysing Fjord (Denmark). Two other clones showed similarity to *dsr* genes of gram-positive thermophilic sulfate reducers (genus *Desulfotomaculum*) and the toluene degrader *Desulfobacula toluolica*, while one was related to *Desulforhabdus amnigena* and *Thermodesulforhabdus norvegica*.

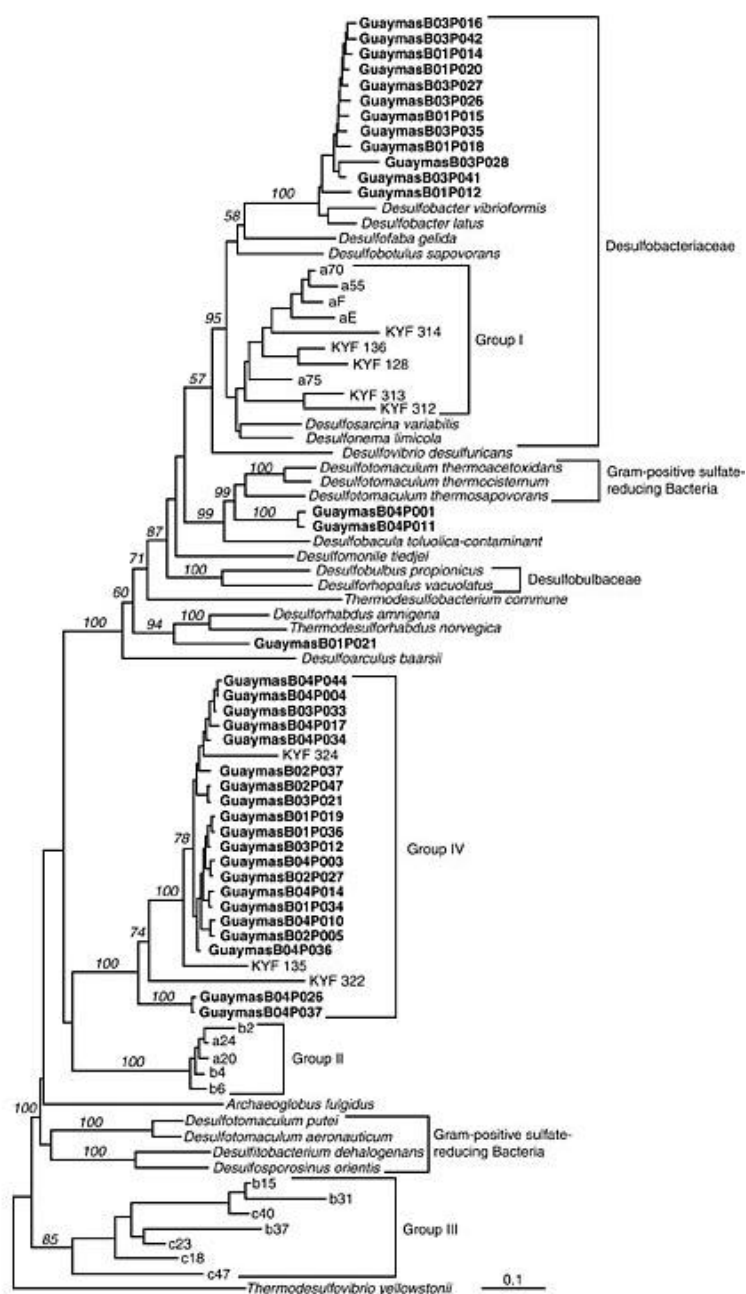


Figure 4. Phylogenetic tree based on the translated amino acid sequences of PCR-amplified *dsr AB* genes from sulfate reducing prokaryotes.

The two-pronged approach of using 16S rRNA and *dsr* clone library sequencing has resulted in a more detailed picture of the sulfate-reducing bacterial community at Guaymas than each of these approaches alone. At Guaymas, the 16S rRNA and the *dsrAB* datasets indicate the significance of members of the Desulfobacteriaceae, most likely the genus *Desulfobacterium*, in the Guaymas sediment. The *dsrAB* dataset also demonstrated the presence of uncultured and unknown major clades of sulfate reducing prokaryotes in the Guaymas sediments that could not be identified by 16S rRNA sequencing.

We also seek to establish linkages between patterns of gene expression that underlie metabolic activity and the formation of biogeochemical gradients. To meet this objective, we must obtain information about global gene expression patterns and detailed information about microbial population structures. We have constructed a custom microarray to examine how the cyanobacterium *Microcoleus chthonoplastes* responds to changes in environmental conditions. The array contains 1090 unique sequences and it has provided information about changing gene expression patterns in response to diel cycling and salinity changes of *Microcoleus* cultures. High and low salt shocks induced quite distinct expression signatures; genes involved in metabolism, signal transduction,

transportation of compounds across membranes, and other cellular processes appear to change their expression levels in response to this environmental stimulus. With knowledge about coordinate gene expression patterns associated with changing metabolic patterns, we will be able to faithfully model fluxes in microbial ecosystems and identify biomarkers for detecting and monitoring biological activity.

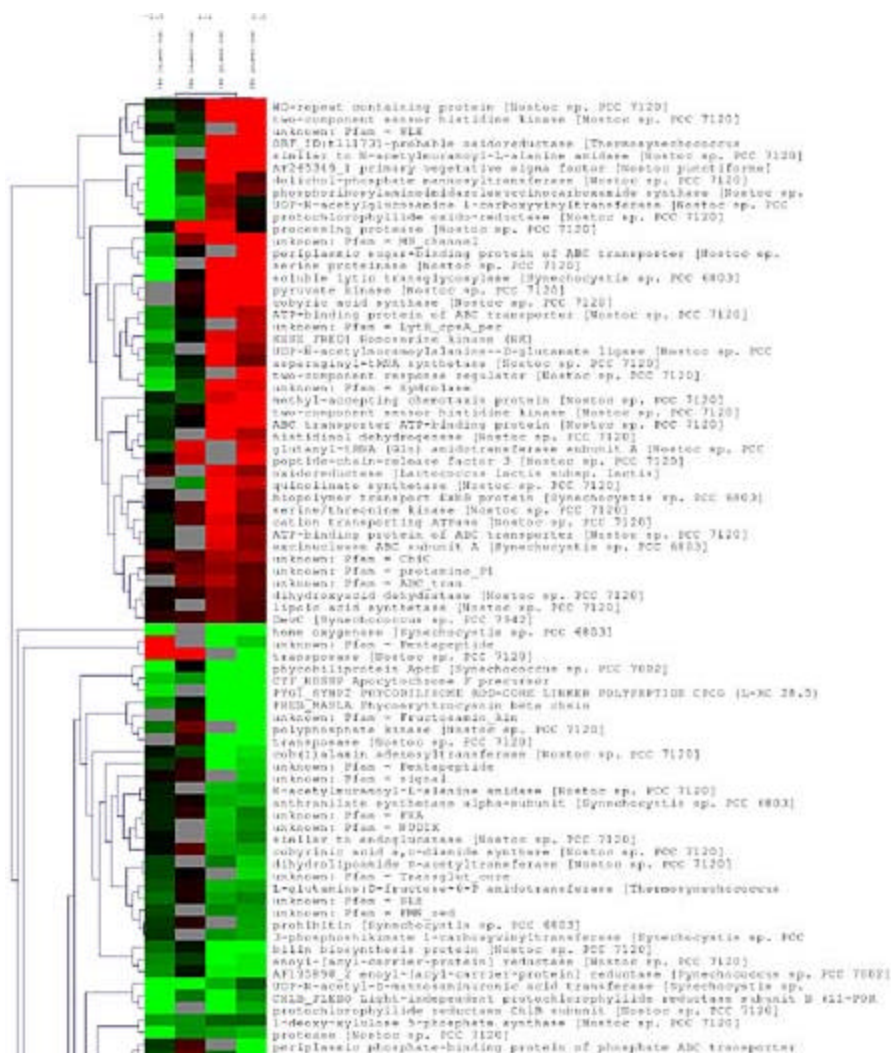


Figure 5. Coordinate gene expression during high and low shock treatments of 1 to 3 hours.

In order to interpret the gene expression data it is necessary to identify the diversity and relative numbers of different kinds of taxa in a microbial setting. Analyses of PCR products from ribosomal RNA (rRNA) coding regions provides a window on the microbial world that has revealed new levels of largely unexplored microbial diversity not represented in laboratory cultures. Unfortunately this low throughput methodology is expensive (typically \$1-2/sequenced template) and sometimes inefficient when a limited number of organisms dominate a microbial population. Deoxyribonucleic acid (DNA) fingerprinting techniques such as denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP) offer higher throughput but little or no taxonomic information, and many fingerprinting studies ultimately rely on DNA sequencing to characterize phylotypes.

We have developed a new, high-throughput method Serial Analysis of Gene Tags (SAGT) that provides estimates of relative frequencies and sufficient phylogenetic information to identify the phylotype of most taxa in an environmental sample. SAGT ligates together the PCR products from orthologous hypervariable regions in rRNA genes to form large concatemers. A single DNA sequencing reaction of a cloned concatemer can include as many as 20-30 orthologous hypervariable regions represented in a population of nucleic acid molecules. In this way, samples loaded onto a 96-channel capillary sequencing machine can provide information about thousands of microorganisms in an analyzed sample. Comparison against a comprehensive rRNA gene database identifies the

taxonomic assignment of individual amplicons in the concatemers. SAGT analyses of bacterial community composition in hydrothermal marine sediments from Guaymas Basin (Gulf of California) are comparable to results of cloning and sequencing of single, full-length PCR products from ribosomal RNA genes of the same microbial community. Both methods identify the same major bacterial groups. The application of this technology will allow us to deeply sample the diversity and relative abundance of microbial populations in any environmental setting.

Education and Outreach.

Our EPO program includes a rich mixture of workshops and courses in disciplines that are relevant to NASA's Astrobiology initiative. Our workshop series includes "Living in the Microbial World", a one-week intensive summer workshop for middle and high school students, focusing on microbial diversity and evolution. "Life and Living in Space", a four-day workshop for high school and community college teachers, convenes in the fall and is offered in conjunction with the Center for Advanced Studies of Space Life Sciences (CASSLS) at the MBL. This workshop combines NASA Life Science topics and areas of Astrobiology content including planetary protection and life detection. We also offered two advanced workshops that target investigators who work in astrobiology related disciplines. The first is the Workshop on Molecular Evolution at the Marine Biological Laboratory directed by Michael Cummings. The Workshop includes a series of lectures, demonstrations and computer labs that span the field of molecular evolution. A distinguishing feature of the course is a state-of-the-art computer laboratory furnished with Linux and Unix servers and workstations for comparative analysis of molecular data. Topics covered in the course include: databases and sequence matching, phylogenetic analysis including Bayesian analysis and maximum likelihood theory, molecular evolution at organismal and higher levels, molecular evolution and development, gene duplication and divergence, gene family organization, evolution of large multigene families, molecular evolution in bioinformatics and comparative genomics.

Our new course Advances in Genome Technology and Bioinformatics, directed by Mitchell L. Sogin and Claire Fraser (President of The Institute for Genomic Research (TIGR)), is a comprehensive, four-week course in genome science that integrates bioinformatics with the latest laboratory techniques for genome sequencing, genome analysis, and high throughput gene expression (DNA microarrays). With a distinguished faculty from major universities and bioinformatics centers, TIGR and the MBL provide instruction that integrates lectures with laboratory exercises both at the computer and in a high technology, high throughput facility.



Figure 6. *The computational biology laboratory for Advances in Genome Sciences and Bioinformatics.*

The major laboratory modules include 1) Genome Sequencing (vector development, library construction, high throughput sequencing technologies, principles of automation using advanced robotic liquid handlers, genome assembly algorithms and closure strategies); 2) Bioinformatics (Gene prediction algorithms, annotation, database

construction and searching, phylogenetics and molecular evolution); and 3) Functional Genomics (DNA microarrays, data analysis). Symposia focusing on Environmental and Evolutionary Genomics, Eukaryotic Microbial Genome Projects, Organelle Evolution and other topics are also part of the program.

In addition to our MBL Astrobiology web site (which is under total revision) we have expanded micro*scope to include a broader range of microbial habitats, more images, more text, enhanced functionality in the form of outlinks and input fields for user contributions. The micro*scope web site (<http://www.mbl.edu/microscope>) is an image-rich resource providing descriptions and pictures of all categories of microorganisms, currently containing thousands of downloadable high-resolution images. The images and accompanying information can be accessed by a number of methods, including by habitat, cell shape, alphabetically by genus name and by hierarchical taxonomic classification. We are developing micro*scope into a central educational repository for students and teachers interested in astrobiology and microbial diversity. The micro*scope website fills the gap in available resources and provides students, teachers and researchers with easy access to high quality digital micrographs and information about diverse microbes, both prokaryotic and eukaryotic. Micro*scope is designed to bring together information distributed at other authoritative sites. It uses software to track the taxonomic location of the user and to initiate searches into remotely located databases or other sites generally available on the web, based on the genus being examined. The list of databases and remote sites to be searched can be customized to return results from only certain selected databases. Our goal is to maintain micro*scope as a resource to assist those interested in gathering information about microbial taxa and in understanding better the diversity, complexity and evolution of the microbial world.

Executive Summary: Michigan State University

Low temperature is a predominant environmental characteristic of interstellar space, asteroids, meteors and of course, our solar system, including most of the planets and their satellites. An understanding of the impacts that low temperature has on the responses and evolution of biological organisms is, therefore, integral to our knowledge of Astrobiology. Toward this end, we are exploring multiple aspects of microbial adaptation to low temperature. One line of investigation -- Genomic and Proteomic Analysis of Permafrost Bacteria -- is to conduct genomic and proteomic analyses of bacteria that have been isolated from the Arctic and Antarctic permafrost. Our basic objectives include identifying genes and proteins that enable permafrost bacteria to inhabit subfreezing environments and determining how genome expression in the permafrost bacteria is affected by low temperature and other environmental conditions associated with the permafrost. We are also interested in conditions that "hitchhiker" bacteria might encounter during travel through space on natural objects or spacecraft. In a second line of investigation -- Bacterial Adaptation to Low Temperature -- we are directly examining, through "test-tube evolution" experiments, how bacteria genetically adapt to low temperatures. The fundamental objective here is to better understand how an organism, with a given complement of genes, can cross niche barriers that are defined by decreasing temperatures. Finally, in a series of "Field Truth" investigations -- Indigenous Bacteria of Arctic and Antarctic Permafrost -- we are exploring the microbial ecology of the permafrost environment and the physiological state of the resident microbial community. This is being accomplished by determining the phylogenetic diversity of the bacterial permafrost population and the metabolic activities present in permafrost soils.

An exciting advance in the Genomic and Proteomic Analysis project is the completion of the genome sequence for *Psychrobacter* 273-4, a bacterial strain that we isolated from 20-40 thousand year old permafrost soil. Completion of this bacterial genome sequence, which is being done in collaboration with the Department of Energy (DOE) Joint Genome Institute (JGI), will represent a milestone in bacterial genome sequencing as no complete genome sequence has been obtained for any psychrotolerant bacterium. Preliminary analysis of the *Psychrobacter* genome, in collaboration with JGI and the bioinformatics group at Oak Ridge National Laboratory, indicates that the isolate encodes approximately 2,140 predicted open reading frames (ORFs), with 11 % of the ORFs being unique to *Psychrobacter*. Furthermore, 42 % of the ORFs had a "hypothetical protein" as the best "hit" in the GenBank database, indicating that the function of a major portion of the *Psychrobacter* genome is yet unknown. A search of the *Psychrobacter* ORFs indicated the presence of potential orthologs for proteins previously shown to be involved in cold adaptation including four Csp cold shock proteins, but a number of genes known to be involved in growth of mesophilic bacteria at low temperature were not found in *Psychrobacter* 273-4 suggesting that it encodes novel cold adaptation genes. Interestingly, 40 transposases from other microorganisms were identified in the *Psychrobacter* isolate suggesting that horizontal gene transfer may have played a significant role in the evolution of its genome.

A fundamental component of the genomics/proteomics project is to determine how expression of the genomes of permafrost bacteria is affected by low temperature and other conditions associated with the permafrost environment. This work is currently focused on *Psychrobacter* 273-4 and *Exiguobacterium* 255-15, a bacterial strain that we isolated from 2-3 million year old permafrost soils. Work to date with the *Psychrobacter* isolate has directly demonstrated that the proteome responds to changes in temperature. In particular, using two dimensional polyacrylamide gel electrophoresis, we found that certain proteins were only present at either cold (4°C) or warm (24°C) temperature. Analysis of the low temperature specific proteins using mass spectrometry indicated that one was novel and that the other three were orthologs of the *Escherichia coli* Csp cold shock proteins. In addition, two of the high temperature specific proteins were found to be novel while a third belonged to the diene lactone hydrolase protein family. To obtain high resolution proteome information for the *Psychrobacter* and *Exiguobacterium* isolates, we have been developing a novel 2-dimensional liquid fractionation method that uses a pH column-based separation in the first dimension followed by separation of the proteins in each pH fraction using nonporous silica (NPS) reversed phase high-performance chromatography (HPLC). The result is a 2-dimensional image of the proteins in the cell as a function of pH versus hydrophobicity. After developing effective cell lysis conditions and appropriate temperatures for protein fractionation, we now have a powerful set of procedures and methods to produce high resolution protein "maps" for the permafrost isolates.

Significant insights have also emerged from the Bacterial Adaptation to Low Temperature project. In studying adaptation of the mesophilic bacterium *E. coli* to low temperature (20 °C), it has been found that the rate of adaptation is independent of previous thermal adaptive history; that is, change in relative fitness or fitness after 2000 generations is no different if the bacteria were previously adapted to 32, 37, or 42°C or an alternation of 32 & 42°C. We have further found that adaptation to 20°C is associated with a significant decrease in fitness at high (40°

C) temperature. Genomic analysis of the 20°C adapted lineages has revealed the existence of several small deletions of the chromosome. The deletions appear to have occurred independently in many of the lineages. Most remarkably, genes at approximately 1.85Mb on the circular chromosome were independently deleted in 6 of the 30 lines examined. Thus, one mechanism of evolutionary adaptation to low temperature appears to involve gene deletion. Moreover, with *E. coli*, the occurrence of deletions in the same region of the genome suggests a functional significance for elimination of a few particular genes. We are in the process of mapping exactly which genes were involved in each lineage and will determine the genes in common that were deleted.

A fundamental objective of the "Field Truth" investigations is to increase our understanding of the phylogenetic diversity of the bacteria that inhabit the Arctic and Antarctic permafrost. In the past, we have examined permafrost under typical subarctic tundra, near the East Siberian Sea, Russia and permafrost of the Beacon Dry Valley, Antarctica. This year we extended our work to the slopes of the active volcano Ploskii Tolbachik (Fig. 1), situated on Kamchatka peninsula in the far-east of Russia. It is one of the largest modern volcanic regions with widespread glaciers and permafrost at high altitudes. This site was chosen because one possibility for liquid water on Mars at shallow depths would be in proximity to subglacial volcanism. Such volcano-ice interfaces could occur beneath the polar caps of Mars today, or even within the adjacent permafrost around the margins of the ice caps.

To examine the microbial communities in this niche, we drilled a series of boreholes at different landscapes and altitudes between 800 and 2600 m a.s.l. (Fig. 2). Frozen (-1 to -2°C) samples extracted from a borehole at 1100m, representing young volcanic interstratified ash, sand and scoria 12 to 16 m thick (from the eruption of 1975-76), contained viable microorganisms and methane up to 1100-1900 µlCH₄/kg soil. We found both psychrophilic and thermophilic microbes in the samples, with the psychrophilic heterotrophs the most numerous followed by psychrophilic methanogens and sulfate reducers. We have obtained enrichment cultures of psychrophilic, mesophilic and thermophilic bacteria of different metabolic types, including acetogens, methanogens, sulfate reducers, feroreducers and spore-formers. The scoria texture of these sites is presumably close to Martian regolith. Such terrestrial volcanic microbial communities in permafrost potentially serve as good exobiological models for testing hypotheses on existing ancient microbial communities.

Last year we reported initial results of determining the diversity of Arctic and Antarctic permafrost bacteria using high throughput 16S ribosomal deoxyribonucleic acid (rDNA) gene sequencing. During the past year, we continued this analysis and determined whether new phylotypes could be isolated after incubating permafrost samples at low temperature (4°C) under both aerobic and anaerobic conditions. The analysis indicated that Arctic surface soils had considerably more phylotype diversity than did Antarctic surface soils and that incubating soil samples at 4 °C resulted in significant changes in the microbial profile in all soil samples tested, indicating that less numerous microbes were alive and could be recovered.

Finally, we have sought to isolate bacterial strains that are related to permafrost bacteria from tropical sites in Puerto Rico. In particular, soil samples obtained from the El Yunque National Forest (hot and moist ecosystem), the Las Cabezas de San Juan Reserve (hot, dry and salty ecosystem) the Guánica State Forest (hot and dry ecosystem) and the Las Carmelitas Cave System were processed for the isolation of Psychrobacter, Exiguobacterium and Arthrobacter strains (we previously isolated Arthrobacter strains from ancient permafrost). Preliminary results indicate that we potentially have isolated relatives of Exiguobacterium and Arthrobacter. The isolation of such strains will provide powerful tools for comparative genomic studies to help identify cold adaptive genes in the permafrost microbes.

Planetary Systems form by collapse of dense inter-stellar cloud cores (Frontispiece). Some stages in this evolution can be directly observed when stellar nurseries are imaged (Figure ES.1), while other stages remain cloaked behind an impenetrable veil of dust and gas. Yet to understand the origin of life on Earth, we must first develop a comprehensive understanding of the formation of our own planetary system.

Dense cloud cores are very cold (10-50 K), and their dust grains are coated with ices comprised of water and organic compounds. Many of these organics have potential relevance to the origin or early evolution of life, if delivered to planets.

The survival of these organics through the violent birth-phase of a star is less certain. Properties of the young star (its mass, spectral energy distribution, whether it formed in isolation or as a multiple star, etc.) play a key role in controlling the evolution of organic material in the proto-planetary disk. The location within the disk is important since the nature and effectiveness of such processing depends strongly on distance from the young star, on distance above the nebular mid-plane, and on time. The ultimate delivery of these primitive organics to young planets and their moons also evolves with time, as the bodies grow in size and as the nebula clears.

We propose to investigate the origin and evolution of organic compounds in planetary systems, and their delivery to young planets.

The proposed research addresses the heart of Goal 3 of the Astrobiology Roadmap:
Understand how life emerges from cosmic and planetary precursors.

The central question is this: Did delivery of exogenous organics and water enable the emergence and evolution of life?

The investigation is divided into four Themes:

Theme 1: *Establish the taxonomy of icy planetesimals and their potential for delivering pre-biotic organics and water to the young Earth and other planets.*

Theme 2: *Investigate processes affecting the origin and evolution of organics in planetary systems.*

Theme 3: *Conduct laboratory simulations of processes that likely affected the chemistry of material in natal interstellar cloud cores and in proto-planetary disks.*

Theme 4: *Develop advanced methods for the in-situ analysis of complex organics in small bodies in the Solar System.*

We seek to better understand the organic compounds generated and destroyed in the interstellar and proto-planetary environments, through observational, theoretical, and laboratory work. We will examine the potential for and limitations to delivery of exogenous pre-biotic organics to planets, examining factors that enhance or restrict this potential.

We will, for the first time, investigate the effect of astrophysical X-rays on the evolution of exogenous organics in proto-stellar disks. We will follow these factors over time, from the natal cloud core through the end of the late heavy bombardment (~4.1 Ga). We will evaluate the possible role of exogenous organic material in terrestrial biogenesis.

The proposed research will significantly improve our understanding of the nature of organics in other planetary systems, the processes affecting them, and the potential for delivering pre-biotic organic compounds to planets.



Click [here](#) for larger image.
Figure ES.1 HST image of NGC 3603 showing the life cycle of material (including carbon) in a star-forming region. A cycle of stellar birth and death leads to the synthesis and evolution of organic compounds. Carbonaceous material ejected from dying stars enters the diffuse medium and then is cycled into dense clouds. The collapse of a dense cloud forms an evolved stellar system (see the Frontispiece for more detail) where these organic compounds can be delivered intact to planetary surfaces and mixed with those produced endogenously. As the lifetime of the evolved system comes to a close, stellar mass loss recycles material to begin the process anew.

The Management Plan: An Integrated Research Approach

The proposed research is interdisciplinary and it involves researchers at multiple institutions. This is both an intellectual asset and an organizational challenge. The effectiveness of a Team is demonstrated when its total output exceeds the sum of its individual parts. We have developed a management strategy that we believe will enable this objective.

- ✍ *Internal collaboration will be enhanced by bridging post-doctoral associates and students across projects within a Theme.*
- ✍ *Theme-Based “Expeditions” will be mounted to ensure that our students receive hands-on experience in techniques used in all Themes.*
- ✍ *Students and post-docs will be encouraged to explore other aspects of Astrobiology at luncheons every two weeks.*
- ✍ *An Executive Scientist will ensure smooth operations of the Node, and timely reporting to NAI Central and to NASA Headquarters.*
- ✍ *An Executive Committee will review the scientific progress and activities, monthly. An independent Board of Visitors will assess progress on an annual basis.*
- ✍ *An Education and Public Outreach Lead will ensure that our E/PO plan is smoothly executed.*

Education and Public Outreach

GSFC and the Minority Institute Astrobiology Collaborative (MIAC) will implement a multifaceted program based on Astrochemistry and focused on organics in the solar system. We will develop curriculum materials, conduct teacher professional development workshops, and bring observational cometary research into middle and high school classrooms. We will support MIAC institutions in the professional development of K-12 educators in under-served communities and build upon existing MIAC, GSFC and UMCP (Deep Impact EPO) programs and educator networks.

Lead Institution Commitment

NASA's Goddard Space Flight Center has long-established scientific expertise in all four Theme areas. The proposed research draws upon large and highly productive ongoing programs in areas of Laboratory Astrochemistry; Planetary Systems research; Interstellar, Stellar, Planetary, and Cometary spectroscopy; and Flight Instrument Development.

The Center has made an advance commitment to the Astrobiology Program by hiring Drs. Jason Dworkin and Michael DiSanti as civil servants, and providing infrastructure support to them. Goddard also plans to fill additional civil service positions in the areas of nebular and cometary chemistry.

The Center has recently devoted an NAS-NRC Resident Research Associateship to Astrobiology and is actively seeking a candidate to fill this position. If our Node is selected, the Executive Scientist will relocate to Goddard. Visiting faculty, post-doctoral associates, and graduate students will be supported to augment the already significant scientific complement at the Center.

Leverage

This proposal heavily leverages the existing research programs of the individual Investigators. Our Investigators have access to advanced laboratories and observatories through their existing institutional arrangements and partnerships.

Principal Objectives

Theme 1: *Organics in Icy Planetesimals: A Key Window on the Early Solar System*

A. Comet taxonomy via specific molecules and isotopes

1. Measure abundances of parent volatiles
2. Measure the ratio HDO/H₂O
3. Measure abundances of chemically related molecules

B. Perform detailed theoretical studies of the molecular chemistry of proto-stellar disks

1. Model infall for specific chemical changes in the major volatile components
2. Determine how trace cometary organics can also be formed at the accretion shock
3. Model interstellar deuterium fractionation as ISM material incorporates into the nebula

C. Model dynamical transport of icy planetesimals in the early Solar System

1. Model organic flux into Oort cloud, terrestrial region, and out of the Solar System
2. Model 1 including giant planet migration
3. Model 2 including the formation of Uranus and Neptune
4. Simulate the growth of grains followed by settling to the mid-plane

D. Determine isotopic compositions and abundances in Lunar breccias

1. Determine the signature(s) of highly siderophilic abundances in Lunar breccias
2. Connect signature(s) to materials exposed to early Solar System processes
3. Determine if the composition of the late influx changed with time
4. Connect siderophilic impactors with those of organic-rich chondrites

Theme 2: *From Molecular Cores to Planets: Our Interstellar Heritage*

A. Study the evolution of material in molecular clouds

1. Map chemical abundances to determine the physical conditions within a molecular cloud
2. Search for new interstellar organic molecules

B. Determine the initial conditions for planet formation

1. Understand the growth of grains prior to and during incorporation in the disk
2. Observe grain growth and document opacity loss as material gets incorporated mm scale bodies
3. Determine the relationship between cometary and interstellar chemistry

C. Connect the X-rays and UV from young stars to formation and destruction of organics

1. Compare abundances of organics around young stars with models and lab simulations
2. Measure the emission and ionization state of molecules near young stars
3. Measure changes in the chemical abundances as a consequence of strong X-ray and UV flaring

D. Search for organic signatures in the IR spectra of transiting extra-solar gas-giant planets

Theme 3: *Organic Material from Laboratory Simulations of Astrophysical Environment*

A. Analyze complex organics in grain-catalyzed reactions

1. Investigate organics in hydration and thermal metamorphism ala various meteorite types
2. Compare results with astronomical observations, meteorites, and Earth-return samples

B. Analyze complex organics in UV, X-ray, electron, and proton processed ices

1. Follow formation and destruction of selected organic compounds in detail
2. Look for the formation of particularly interesting biological molecules
3. Compare results with astronomical observations, meteorites, and Earth-return samples

C. Analyze more complex simulations

1. Combine materials and techniques of 3A and 3B
2. Investigate organics in residues in various aqueous environments
3. Follow the reactions of residues mixed with grains
4. Follow the reactions of residues induced by additional ion or photon processing

Theme 4: *Advanced Analysis of Primitive Material*

A. Determine how to measure the history and the chemical state of organics in situ

1. Evaluate a number of possible chromatographic mass spectral techniques
2. Compare bulk pyrolysis and laser and ion beam volatilization for this evaluation (4A.1)

B. Evaluate, minimize, and manage thermal perturbations to Earth-return samples

1. Determine how to preserve the structure and isotopic composition of relevant organics
2. Optimize method to determine the original composition of compounds before heating

C. Utilize lab analogs to develop and calibrate instruments

Executive Summary: Pennsylvania State University

The Penn State Astrobiology Research Center (PSARC), created five years ago as part of the NASA Astrobiology Institute, is composed of 16 (Co-)PIs and their research teams from The Pennsylvania State University (13), The University of Pittsburgh (2), and SUNY Stony Brook (1). The investigators represent a wide range of disciplines: geochemistry (Michael Arthur, Susan Brantley, Rosemary Capo, Lee Kump, Hiroshi Ohmoto, Martin Schoonen, and Brian Stewart), paleontology (Mark Patzkowski), atmospheric chemistry (Jim Kasting and Robert Minard), geomicrobiology (Kate Freeman and Chris House), evolutionary genomics (Blair Hedges and Masatoshi Nei), and biochemistry and microbiology (Jean Brenchley and Greg Ferry). The proximity of all members has enabled close interaction and a variety of collaborative research, teaching, and public outreach programs. During the fifth year, PSARC has supported all or part of the research/education/PO activities carried out by 142 persons (16 (Co-)PIs, 22 research associates and postdoctoral fellows, five research assistants, two technicians, 49 graduate students, 37 undergraduate students, and five staff in administration/IT/EPO).

The primary research goal of PSARC over the past five years (under the theme Coevolution of the Earth and Life) has been to increase understanding of the connections between the rise of major life forms during the early history of Earth (between ~3.8 and 0.5 billion years ago) and the evolution of the environment (especially atmospheric O₂, CO₂, and CH₄). This goal has been pursued primarily from multidisciplinary and multidimensional research focused on the following seven topics (Tasks):

1. Environment of prebiotic Earth and the origin of life
 - a. Experimental approach (Schoonen)
 - b. Prebiotic chemistry of hydrogen cyanide (Minard)
2. Biochemistry of Archea and Bacteria
 - a. Enzymes of ancient metabolic pathways (Ferry)
 - b. Biochemistry of psychrophilic organisms (Brenchley)
 - c. Microbe-mineral interactions (Brantley)
3. GEOPULSE: Gene Expressions Observations for Planetary Life Study (House, Ferry, Freeman, Brantley)
4. Timescale for the evolution of life on Earth: Molecular evolutionary approach (Hedges and Nei)
5. Evolution of atmospheric O₂, climate, and biosphere (Kump, Kasting, Freeman, Capo, Stewart, and Ohmoto)
6. Neoproterozoic variations in carbon and sulfur cycling (Arthur)
7. Causes and consequences of the diversification and extinction of metazoans (Patzkowsky)

Progress in each of the above topics is reported separately in the following pages. Excellent progress has been made in all phases of our research projects. The new discoveries made and the new theories developed from these investigations during the fifth year have been presented in 34 published papers (including those in press) and 20 papers (submitted, in review, or in revision) in refereed journals and book chapters, and 71 abstracts presented at international and national meetings.

Highlights

Topic 1: Prebiotic Environment

Martin Schoonen's group has conducted a series of photochemical experiments using pyrite, and suggests pyrite-induced OH radical formation may have placed an important constraint on the stability of biomolecules, such as ribonucleic acid (RNA), on early Earth. Protective mechanisms, such as encapsulation of biomolecules by lipids, may have been a prerequisite to escape decomposition via OH radical attack.

Robert Minard has synthesized H¹³C¹⁵N polymers under a variety of conditions, and analyzed their structures using solid-state nuclear magnetic resonance (NMR). Based on these data, Minard suggests the HCN polymer structural model provides a mechanism for the formation of heteropolypeptides on the primitive Earth.

Topic 2: Biochemistry of Archea and Bacteria

Greg Ferry has discovered a novel flavoprotein family (archaeoflavoprotein) that is unique to the Archaea domain of life.

Jean Brenchley's group has demonstrated that a wide, and perhaps surprising, diversity of organisms have survived entrapment in a Greenland glacier for over 120,000 years. They have analyzed nearly 800 isolates, grouping them into taxonomic and phylogenetic categories.

Susan Brantley's group has found that Fe (II) released from goethite by siderophore-producing soil bacteria has a dFe value -1.6 ‰ relative to goethite. They have also found that nitrogen-fixing bacteria may secrete molybdenum-complexing ligands to solution in order to extract Mo for nitrogenase: for example, *Azotobacter vinelandii* secretes aminochelin when grown in Mo-deficient but Fe-replete conditions.

Topic 3. Gene Expression for Planetary Life Study

Using whole genomic analysis, Chris House's group has deduced that sulfur reduction is the most geologically plausible for the base of the Archaea. They have also found that: (a) *Methanosarcina acetivorans* have surprisingly high oxygen tolerance, allowing them to grow even at about 1% oxygen; (b) *Archaeoglobus* does not oxidize methane in spite of the similarity of *Archaeoglobus* to ANME-1 and ANME-2; and (c) there are conditions that increase trace methane oxidation by methanogens, providing some insight into how anaerobic methane oxidation occurs.

Topic 4. Evolutionary genomics

Blair Hedges's group has found, using molecular clock studies of large numbers of genes (50-200), that plants, animals, and fungi diverged about 1.5 billion years ago, with living groups in each kingdom diverging relatively soon thereafter. This is as much as one billion years earlier than indicated by the fossil record.

Masatoshi Nei, a member of the National Academy of Science, was awarded the 2002 International Prize for Biology in the field of "Biology of Evolution" for his contributions in evolutionary genomics.

Topic 5. Evolution of atmospheric O₂, climate, and biosphere

Lee Kump's group has found from numerical modeling of microbial mats that O₂ levels in Archean cyanobacterial mats may have exceeded modern atmospheric saturation values by a factor of 2-3, as modern mats do during afternoon hours. This conclusion is independent of the oxygen or sulfide content of the overlying water. From carbon isotope analyses of Paleoproterozoic carbonate, Kump also suggests that pelagic marine organisms in the Paleoproterozoic (1.8 billion years ago) apparently were modifying the nutrient and carbon chemistry of the ocean in much the same way and to a similar extent as modern organisms do. Kump also suggests the banded iron formations in the Neoproterozoic "Snowball Earth" period may have been the result of high iron fluxes from mid-ocean ridges, which were enhanced by a sea-level change and the low-sulfate oceans.

Jim Kasting's group, based on photochemical modeling, suggests that: (a) enhanced methane concentrations could provide a plausible explanation for the extended warmth of the Mid-Proterozoic Era, 2.2-0.8 Ga; and (b) ozone and methane might be observed simultaneously in a Mid-Proterozoic-type terrestrial planet atmosphere. The simultaneous presence of O₂ (or O₃) and reduced gases is considered the firmest remote evidence for extraterrestrial life.

Based on molecular analyses of organic matter from sedimentary rocks of late Archean age, Kate Freeman's group suggests diverse life already existed in the Late Archean. There is evidence for all three domains existing at that time. Isotopic and stratigraphic data reveal ecological differences associated with lithofacies. Shallower facies were more oxygenated, a phenomenon that is reflected in the recorded microbial processes. Deeper waters were largely anoxic, although there is evidence for redox cycling between elemental sulfur and sulfide. Isotopically enriched tetramethyl- and trimethylbenzene released via pyrolysis can be linked to inputs from green sulfur bacteria in ancient (Cretaceous) lake deposits. This may extend the available biomarkers for this source of organic matter.

Rosemary Capo and Brian Stewart's group has found that the Sm-Nd isotope system can be used to evaluate the pedogenic mobilization and possible later remobilization of geochemical tracers in ancient soil profiles. Soil carbonate is a significant repository for rare Earth elements (REE), an important paleoatmospheric tracer, and must be considered in working out the REE budgets of ancient soil profiles formed under arid conditions. They further suggest the REE patterns of the ~3 Ga Steep Rock paleosol, Canada, reflect pedogenic processes.

Hiroshi Ohmoto has organized the Archean Biosphere Drilling Project (ABDP), an international collaborative research project under the Astrobiology Drilling Program (ADP). The drilling, which began in the Pilbara district, Western Australia, in June 2003, has already revealed unequivocal evidence that the hematite (ferric oxide) crystals in the 3.46 Ga Marble Bar chert/jasper sequence were not formed by the modern oxidation of ferrous-rich carbonate (siderite) as postulated by many previous investigators, but instead formed by the mixing of Fe²⁺-bearing submarine hydrothermal fluids and oxygenated local seawater when the rocks accumulated on the ocean floor. This is important evidence suggesting the oceans and atmosphere were already oxygenated 3.46 Ga ago. The ABDP has also recovered a large amount of black shales, containing remnants of microbes that lived in the Archean oceans. The mineralogical and geochemical investigations (e.g., C, N, and S isotopes; rare Earth elements) by Ohmoto's group on Archean shales and banded iron formations from the Abitibi district, Canada, the Pilbara-Hamersley district, Australia, and other places have also revealed that the redox structure of the Archean oceans was probably identical to that of modern oceans: globally oxic oceans with locally developed anoxic basins that sustained complexed ecosystems. Based on analyses of thermodynamic data and carbon isotope data on siderite (FeCO₃), which is abundant in banded iron formations older than ~1.8 Ga, Ohmoto and Watanabe suggest the CO₂ level of the Archean atmosphere was at least 100 times greater than today, implying CO₂, rather than methane, was the major green-house gas in Archean, as well as in the later geologic time.



Figure 1. *The first drilling site of the ABDP.*



Figure 2. *Hiroshi Ohmoto examines the first core with Bruce Runnegar (incoming NAI Director) at the site.*

Topic 6. Neoproterozoic carbon and sulfur cycles

Based on sulfur isotope analyses of trace sulfate in carbonates, Mike Arthur's group suggests the seawater sulfate concentrations in the mid- to late-Proterozoic were perhaps 10% of those at present. These low concentrations led to profound changes in ocean and atmospheric chemistry, ultimately producing near-global glaciations.

Topic 7. The Ordovician extinction of metazoans

Mark Patzkowski's group has made significant progress in understanding the environmental causes of the Late Ordovician glaciation and the evolutionary consequences of the Late Ordovician mass extinction. Based on numerical modeling, they suggest the $p\text{CO}_2$ level was < 8 times the present atmospheric level (PAL) when the glaciation began and > 10 PAL when it ended.

Executive Summary: Scripps Research Institute

What is life, the requirements for its origins and evolution, and how living systems may be identified elsewhere in the universe are some of the most fundamental questions in astrobiology. Under the auspices of the Scripps Research Institute, a multi-institutional research team has been assembled to explore a variety of interdisciplinary experimental approaches to self-reproducing molecular systems and Darwinian chemistry. Through the design and study of diverse and novel chemical systems in the laboratory, we seek to garner a better understanding of life and its origins.

In the past year our team has continued to make significant discoveries and progress in several areas of research. Benner's group at the University of Florida has established a multidisciplinary research program that addresses issues relevant to the Astrobiology from several distinct but interrelated perspectives. They have hypothesized that genetic molecules in water will contain a repeating charge, universally in the galaxy. They have completed studies on a series of molecules that test this hypothesis, a step towards developing tools to detect universal biosignatures in NASA missions. Furthermore, a key step towards a synthetic biology involves the generation of artificial genetic systems that can be copied, and then have their copies copied. They reported a six letter genetic alphabet has supported a polymerase chain reaction. A long-standing debate in the history of life asks the temperature at which primitive microorganisms live. Benner's group have resurrected proteins from ancient bacteria, studied their behaviors at different temperatures, and concluded that these bacteria lived at temperatures between 60 and 70 °C. Finally, considering that the most abundant solvent in the solar system is not water, rather supercritical mixtures of dihydrogen, helium, and molecules such as methane and ammonia, Benner and coworkers have begun exploring, from a theoretical perspective, how organic molecules might behave differently in such environments. Ellington's laboratory at the University of Texas-Austin is interested in generating novel self-replicating biopolymers in order to better identify self-replicating molecular ensembles that may be encountered on other planets. During the past year Ellington's group has designed a cross-catalytic amplification system based on the fast and efficient deoxyribozyme. In this system complementary deoxyribozyme cleavages are inactivated by circularization. Linearization results in activation of the deoxyribozyme and leads to the initiation of a cascade of cleavage reactions that display exponential growth kinetics. This system represents the first in vitro system capable of exponential growth in the absence of protein enzymes. Ongoing in vitro selection experiments using larger and more diverse pools are expected to allow elucidation of some of the parameters surrounding the evolution of sequence and function in exponential replicating systems. The goal of Ghadiri's research program at the Scripps Research Institute is to design, discover, and understand the primary factors responsible for directing self-organization of inanimate molecules into the animate chemistry of living systems. Ghadiri's and coworkers' approach has been to rationally design and recreate various forms of autocatalytic peptide networks in the laboratory and study how the interplay of molecular information and nonlinear catalysis can lead to self-organization and expression of emergent properties. Recently they have completed the design of the first de novo "small world" synthetic chemical network based on template-directed coiled-coil peptide fragment condensation reactions. The self-organized chemical network is composed of nine nodes and 21 edges including three autocatalytic processes. Their studies indicate for the first time that relatively complex networks can be rationally designed and constructed and its properties experimentally analyzed in order to gain insights into the dynamics of complex nonlinear chemical networks. This approach is expected to allow rigorous experimental and theoretical analysis of network architecture and aid in better understanding of factors contributing to system self-organization and display of emergent phenomena in living systems. Rebek and coworkers have continued their pioneering efforts in the design of novel abiotic molecular replicators and proto-cells. They have continued to explore synthetic systems in which compartmentalization leads to nonlinear, autocatalytic behavior. The compartments are reversibly formed capsules in which small molecular guests are temporarily surrounded by larger molecular hosts. They represent an extreme form of molecular recognition -- molecules within molecules. The complexes are held together by weak intermolecular forces and their lifetimes vary from milliseconds to days. This range allows their applications as nanometric reaction chambers, as means to stabilize reagents and as spaces where new forms of stereochemistry can emerge. The capsule provides a mechanical barrier that imposes restrictions on the motion of guests held inside. The primary goal of the Switzer laboratory is to synthesize Alternative Nucleic Acids (ANAs) to attempt the optimization of polymer structure subject to the constraints of prebiotic availability, template-directed reproduction, replication conservative mutation, and fitness. Switzer and coworkers have designed and successfully evaluated a novel metallo-base-pair for deoxyribonucleic acid (DNA) that has greater thermal stability than natural base-pairs. This discovery expands the bounds of environmental conditions suitable for the transfer of genetic information by increasing the fitness of nucleic acid-like molecules in thermal environments, while also adding another orthogonal base-pair to the "genetic alphabet." In an ongoing study, Switzer's laboratory has been studying a new peptide nucleic acid that transcends previous known shortcomings of this molecular chimera of nucleic acids and peptides,

opening the way to the creation of long polymer chains and highly functional proto-biopolymers that could serve as transitional polymers on the way to the present DNA/ribonucleic acid (RNA)/protein world.

Executive Summary: University of Arizona

We plan to carry out three investigations concerning the astronomical constraints on the possible origins of life in the Universe.

Our first module will focus on astrochemical investigations concerning the building blocks of life. Under the leadership of L. Ziurys, we plan to: (i) undertake laboratory studies to determine signatures for pre-biotic compounds that might exist in the interstellar medium, with special emphasis on ribose; (ii) initiate an observational search for these gas-phase molecules that are important for life using millimeter and sub-millimeter radio telescopes to which we have access; and (iii) conduct theoretical studies into the stability of complex organic molecules in the gas phase ISM and the evolution of their abundances in circumstellar disks in order to interpret the broader implications of our findings for the possibility of life elsewhere in the Universe. This module will address Goal #3 in the NAI revised roadmap.

Our second module will focus on the formation and evolution of habitable worlds under the leadership of S. Strom and J. Najita (NOAO). We plan to: (i) undertake an observational program to learn when, where, and how frequently planets form around young stars to provide the possibility for constraints on habitable planet formation; (ii) conduct an observational characterization of circumstellar environments that give rise to life and initiate a theoretical modeling program to determine the frequency of giant impacts as traced through circumstellar dust disk evolution, connecting the evolution of dusty disks around sunlike stars to the history of our solar system; and (iii) investigate the time evolution of the UV/x-ray flux of young solar-type stars from the protostellar phase through the epoch of terrestrial planet formation to mature planetary systems. The investigation will also include studies of stars that have evolved further than the Sun, so as to predict the future variability of the Sun. This module addresses Goal #1 of the NAI Revised Roadmap, as well as aspects of #4 and #6.

Our third module, under the leadership of R. Angel and P. Hinz and is aimed at the characterization of planetary systems. We plan to: (i) initiate an observational program aimed at the direct detection and characterization of astrophysically relevant extra-solar giant planets (EGPs) to determine their frequency around solar-type stars and characterize their composition through spectroscopy; (ii) initiate a theoretical study of giant planet atmospheres extending current modeling efforts down to 0.1 MJupiter as a first step in understanding the detectability of biosignatures in planet atmospheres with liquid water present; and (iii) extend observational work on the observed earthshine spectrum to near-infrared wavelengths to close a gap in our understanding as well as initiate a monitoring program. This module addresses goals #1 and #7 in the NAI Revised Roadmap.

Our special emphasis on the revised roadmap Goal #1 arises because of current lack of understanding of how the Solar System arose, and how it fits with the many other planetary systems which are being discovered, and which have substantial differences from the Solar System. Our goal is to set a better, more appropriate starting point for consideration of the origin of life, and to start to explore the question of whether Earth is or is not rare.

Our plans to strengthen the astrobiology community revolve around the creation of the Laplace Center for interdisciplinary astrobiology studies and the Astrobiology Winter School to be held annually here at the University of Arizona. The Laplace Center will be an interdisciplinary program (IDP), a standard system for interdisciplinary research and teaching at UA. It will serve as a focus for our interdisciplinary research efforts and strengthen the growing ties between the Departments of Astronomy, Planetary Sciences, NOAO, Geosciences, Chemistry and Biochemistry. Future plans call for including also Microbiology and Ecology and Evolutionary Biology as well as the Tree Ring Laboratory, Optical Sciences, and parts of the Medical College.

The Center will host 2-3 extended visitors per year and organize meetings to increase scientific interactions across the boundaries that exist within the College of Science. The Winter School will train up to twenty students per year over the period of performance of this proposal. We plan to host visiting faculty for up to two months per year from partner NAI node institutions as well as 10 graduate students for a four-month curriculum in the origins of life. In collaboration with colleagues throughout the College of Science we will offer two interdisciplinary courses for graduate credit that focus on the boundaries between the disciplines and take advantage of the unique observational and laboratory facilities on the University of Arizona campus.

Our efforts to “Strengthen the Astrobiology Community” will include production of first rate scholars in areas important to the future success of the field. In addition to extant interdisciplinary graduate programs at the

University of Arizona such as those in Planetary Science (Astrobiology Minor), Chemistry (Astrochemistry emphasis), and Optical Sciences (various joint degree programs) we will introduce an Astrobiology minor as part of the IDP for the departments within the College of Science.

A particularly innovative part of this proposed project is for a significant and integrated education and public outreach component led by Tim Slater. Because a comprehensive program to improve the public's understanding of this interdisciplinary science requires targeting schools, we will work directly with secondary school teachers on improving their understanding of the myriad of underlying concepts surrounding the search for other worlds. Initially, we plan to conduct systematic studies of the understanding and beliefs about the scientific search for other worlds held by K-14 students, teachers, and the general public. In addition to contributing to the scholarly literature base of science education, a detailed understanding of the existing notions and attitudes people have about this interdisciplinary science are crucial to designing the most effective education and public outreach programs. This will be accomplished through a systematic series of surveys, interviews, and carefully monitored instructional interventions. The results will be disseminated through journal articles and presentations at professional education conferences.

Finally, we also plan to help other graduate students and university faculty supported by this program to become informed about the reasoning difficulties K-12 students, teachers, and the general public have with understanding the search for other worlds. If research scientists have an appreciation for the specific parts of this science that people find difficult, they will be better able to communicate the exciting results and enhance the public's attitudes toward supporting this endeavor. This will be accomplished by conducting frequent workshops and contributing scholarly papers at professional science conferences as well as regularly contributing to the program's seminar series. In support of these efforts, the team will undertake the creation and dissemination of an astrobiology public speakers toolkit and a dynamic FAQ (frequently asked questions).

Overview

Mars is an exciting, and comparatively accessible target for astrobiological studies aimed at detection of current or past extraterrestrial life. We will analyze the evolution of the Martian hydrosphere and surface topography to understand the history of water distribution and investigate atmospheric processes that may have contributed to a UV shield. Our goal is to identify the types of sites on Mars that experienced long-term fluid flow and may be, or have been, conducive to life. We will characterize biomes that develop in analogous Earth environments, conduct experiments to determine limitations for life in these habitats, and identify features that constitute indicators of life. We propose robot-based sampling and in situ analyses of terrestrial sites so as to develop methods for dealing with the challenges of remote geomicrobiological investigations. Our work will provide constraints for selection of optimal sites for future Mars exploration and methods for sample analysis, and ultimately will be relevant to the question 'did life evolve elsewhere in the universe'.

Habitat Constraints from Mars and Modeling

Early microbial life probably originated on Earth in environments characterized by redox disequilibria. Habitats may have developed on Mars in redox gradients between reduced basaltic rocks and oxidized fluids and/or gases. Element cycling driven by fluid flow through such redox gradients could underpin (or could have underpinned) a substantial biosphere. We will analyze Mars planetary evolution to develop models for the timing and scale of hydrosphere development and subsurface water circulation. We will couple these hydrosphere models to geomorphological models based on terrestrial field site analyses and experimental geomorphological studies to allow detailed interpretation of Mars surface features. This will permit analysis of the history, form, and timing of fluid flow events that shaped the planetary surface and determination of the factors that control them. In parallel, we will explore atmospheric processes that could have contributed to a UV shield and conduct spectroscopic studies to constrain Mars surface mineralogy.

Habitat Constraints from Earth

Studies of chemoautotrophically-based ecosystems will focus on terrestrial aqueous environments in basaltic rocks similar to those at the Martian surface. Hydrology, geomorphology, spectroscopy, and geomicrobiology research will begin at sites where groundwater discharge in basaltic andesites or basalts is generating channels with features similar to those on Mars. These springs appear to offer the best chance of sustained water flow and protection from UV radiation. Initially, our studies will be conducted at cold and warm springs associated with basaltic rocks in dry, cold desert environments in Oregon and Idaho. We will refine our choice of study sites as our understanding of Mars' surface improves.

Abundant, redox-active species such as iron and sulfur represent potential energy sources for possible Martian life at springs in basaltic rocks. Recent microbiological studies, geochemical calculations, and experiments indicate that the kinetics of both Fe-silicate and Fe-sulfide mineral dissolution reactions are fast enough to sustain significant biological populations. We will characterize currently poorly understood microbial habitats in the near subsurface in terms of their population structure, aqueous geochemistry, mineralogy, and isotopic signatures in order to determine the form of the record life might leave in similar Martian systems.

Laboratory Constraints for Habitat Development and Biosignatures

Results of in situ analyses of terrestrial ecosystems will be paralleled by laboratory-based studies that will explore the ranges of temperature, concentration, and pH consistent with life in these habitats. Biochemical analyses will explore the factors that set these limits. We will analyze the structure, elemental and isotopic composition, microstructure, morphology, and distribution of minerals generated by, or impacted by, life in basaltic-rock hosted systems so as to develop and test potential new biosignatures. Parallel inorganic experiments will be conducted in order to resolve non-biological features and to examine changes in mineralogical biosignatures with time. As yet unstudied isotopic characteristics of Martian meteorites will be determined in order to provide baselines for isotopic biosignatures. Similarly, work on the isotopic evolution of the atmosphere will establish the magnitude and form of non-biological isotope fractionations. Application of state-of-the-art methods for analysis of Martian, and Mars-like rocks will yield procedures that will be useful for future analysis of samples returned to Earth or encountered during

remote analysis on the Martian surface. Our results will contribute to selection of sites on the Martian surface with the highest potential for future detailed in situ investigations.

Our Team

Our goal is to create a highly interactive, focused NAI team to address a well-defined set of problems. The necessary interactions will be facilitated by close geographic proximity of most team members. Five of the 10 scientific team members are at UC Berkeley, one is at NASA Ames, and one in Palo Alto. The three other PIs have essential expertise for study of difficult to cultivate Fe-oxidizing neutrophiles and in situ measurements. All three non-Bay area coPIs have collaborated with the PI on a NASA-funded seed project preliminary to this proposal. Communication between all PIs will be promoted through work on common sites and processes, shared goals, and virtual (internet-based conferencing) and traditional group meetings.

Our group includes members with strong, integrated field and laboratory-based research programs and experience with study of a diversity of natural environments. Several team members are expert in the development and deployment of state-of-the-art analytical methods (e.g., isotopic analyses, microsensor measurements) to interdisciplinary problems. Our group also includes a robotics engineer and scientists familiar with ancient and recent Mars planetary history. The NAI support will be essential to facilitate the new interactions between hydrologists, geomorphologists, geomicrobiologists, chemists and engineers that are needed to meet the project goals.

Education and Public Outreach

The topics of life on Mars, life in extreme environments, and extraterrestrial exploration easily capture public attention. Our group will use the broad appeal of these subjects to create educational materials designed to foster interest in science, especially geology, chemistry, and biology. Educators from the University of California Berkeley's Lawrence Hall of Science (LHS) will work closely with the BIOMARS team to develop, field-test, and implement materials that incorporate key project concepts and emphasize the interdisciplinary nature of space exploration. LHS is a public science center that is world-renowned for development of high quality middle and high school science curriculum materials based on current research and understanding of how students think and learn. Educational materials will be disseminated through the use of the LHS infrastructure, and its well-established national and international network of educators.

Summary of Personnel

Principal Investigator:

Dr. Jillian Banfield
University of California, Berkeley

Co-Investigators:

Dr. Janice Bishop,
SETI Institute

Dr. Kristie Boering
University of California, Berkeley

Dr. Donald DePaolo
University of California, Berkeley

Dr. William Dietrich
University of California, Berkeley

Dr. David Emerson
ATCC/George Mason University

Dr. George Luther
University of Delaware

Dr. Michael Manga
University of California, Berkeley

Dr. Eric Roden
University of Alabama

Dr. Mark Yim
Palo Alto Research Center

Education and Public Outreach:

Kevin Cuff
Lawrence Hall of Science

Dr. Herbert Thier
University of California Berkeley

Executive Summary: University of California, Los Angeles (UCLA)

Building on seven years of experience and accomplishments in the planning, implementation, and operation of the NASA Astrobiology Institute, UCLA lead team members propose to embark on another five-year plan of research, education, and public outreach in astrobiology that makes full use of the remarkable sets of talents and tools that have been assembled for these purposes over the past five years. The UCLA Center for Astrobiology (CAB) is one of five centers of the UCLA branch of the University of California-wide Institute of Geophysics and Planetary Physics (IGPP) and one of the eleven founding lead teams of the NASA Astrobiology Institute (NAI). Research, education, and public outreach activities of the Center are aimed at the long-term questions and goals of astrobiology, namely: How do planetary processes give rise to life? How are planets formed? Are there habitable planetary bodies outside our Solar System? When and how did life appear on Earth and, possibly, Mars? What are the biological innovations that allow life to climb to complexity on planetary timescales?

The team consists of more than 50 investigators. If this proposal is successful, UCLA will contribute ~ \$1.3 million to match the \$7.2 million requested of NASA. In addition to the University contribution, and in the spirit of the Cooperative Agreement, CAB participants bring to the project a wide range of skills and resources. Our plan calls for distributing these resources in ways that maximize the scientific and educational output beyond that which would be achieved by the investigators on an individual basis. We will use a method of strategic allocation of funds to promote interdisciplinary research that would be difficult to carry out without the existence of the Center. Examples of interdisciplinary research planned for the next five years include: collaborations between astronomers, atmospheric chemists, and cosmochemists that are leading to entirely new theories for the way that our Solar System evolved; the bringing together of paleontologists, atmospheric chemists, and isotope geochemists to study atmospheric evolution on ancient Earth; integration of advanced orbital and rotational dynamical calculations into studies of mass extinctions and the climate history of Mars; and collaborations between paleontologists and microbiologists with the goal of understanding the origins of eukaryotic life. In some cases the impetus for interdisciplinary work proposed here has come from merely bringing workers with disparate backgrounds together at CAB meetings. In other cases CAB funds will allow individuals to have access to facilities they might not have been aware of, much less had access to, previously. In still other cases the support of a student or a researcher will allow an investigator to participate in transdisciplinary studies that they would not otherwise engage in. In all cases, the new avenues of research will further the goals of astrobiology as a discipline and will be possible through the activities of the UCLA Center for Astrobiology in the coming years.

The title of this proposal reflects the breadth of research to be carried out if it is funded. Our investigators and collaborators have expertise in astronomy, cosmochemistry, geology, geobiology, geophysics, geochemistry, paleontology, planetary science, microbiology, organismic and molecular biology, and, of course, astrobiology. They represent UCLA's Departments of Atmospheric Sciences, Chemistry & Biochemistry, Earth & Space Sciences, Mathematics, Microbiology, Immunology & Molecular Genetics, Molecular, Cell & Developmental Biology, Organismic Biology, Ecology & Evolution, Physics & Astronomy, and the Molecular Biology Institute, as well as many other off-campus institutions.

The team assembled for this proposal includes a comparatively large (relative to our previous group) number of astronomers and cosmochemists. This reflects our goal of assuming a leadership role in helping to strengthen the astro component of astrobiology, as suggested by the National Research Council's Committee on the Origins and Evolution of Life in their 2002 assessment of NASA's astrobiology program. While astronomers may not work directly with microbiologists, CAB astronomers will work with the cosmochemists in ways that they have not before, and the cosmochemists in turn will work with geochemists who at the same time will collaborate with microbiologists in novel ways. In this fashion, progress in a given area will be informed by work in even the most dissimilar discipline when the need arises. Such a chain of informed inquiry is crucial to a field that seeks to define the range of possible habitable environments in all planetary systems and to match this range of habitats with the full spectrum of possible forms of life.

The UCLA astrobiology research plan for the next five years focuses on four themes: (1) Extrasolar Planetary Systems; (2) Habitability of Planets and their Satellites; (3) Earth's Early Environment and Life; and (4) Evolution of Biological Complexity. UCLA lead team members have considerable experience in each of these areas. Their accomplishments range from the recent detection of asteroids and comets around another star to identifying the earliest evidence for life on Earth. Some members of our team have been at the forefront of technological advances in measuring isotope ratios at microscopic scales in natural materials, capabilities that are central to many of the studies planned as part of this program. Others are Principal Investigators or Co-Investigators on exciting new

astronomy missions that are equally important to our proposed research program. Still others are leaders in the fields of microbiology and paleontology.

How typical is our Solar System? This is a key question that arises when one attempts to gauge the likelihood for life elsewhere in the Galaxy. UCLA lead team members are beginning to answer this question by studying both extrasolar planetary systems and the Solar System itself. The most successful method for detecting planets beyond the Solar System has been to measure the wobbles exerted by planets on their stars. The method, by its very nature, is biased towards detecting giant planets in close proximity to the star. It is generally recognized that such planetary systems -with giant planets much closer to their stars than Jupiter is to the Sun -are unlikely to harbor life. Members of the UCLA team are developing and applying methods more suitable for detecting planetary systems that more closely resemble the Solar System. The underlying assumption is that these systems are more likely to have Earth-like planets. Even where planets can not be detected, our group proposes to search for indirect evidence for planet formation. Rock and ice debris around some stars may signify the existence of comets and asteroids, the precursors to, or vestiges of, rocky planets that could resemble Earth. We will do all of this with access to the finest tools, including the Keck observatories and new platforms for observations from space (SIRTF) and in the stratosphere (SOFIA) that permit imaging with infrared wavelengths with minimal interferences from air, as well as various radio telescope facilities.

Looking over the shoulders of the astronomers are those members of our team studying meteorites and the story these rocks have to tell about the origin of the Solar System. By virtue of their involvement in the Center for Astrobiology, the cosmochemists and astronomers are working together to discover the ways in which water might be incorporated into Earth-like planets, and whether photochemistry in the disks of dust and gas surrounding young stars is instrumental in determining the nature of the organic and inorganic building blocks of planets. This synergistic activity has already resulted in proposals for new astronomical measurements that would not otherwise have been made.

While hunting for extrasolar planetary systems and comparing them to the Solar System is requisite for gauging the prospects for life elsewhere, it is not sufficient. It is also necessary to define those objects that are habitable. Our team is addressing this issue by studying the factors that might control the habitability of the icy moons of Jupiter, the time-dependent climate of Mars, potential links between the dynamics of rocky planets and their long-term habitability, the influence of orbital dynamics on the prospects for Earth-like planets in extrasolar planetary systems, and the role that impacts play in survival and evolution of life.

Manifestations of Earth's earliest life are controversial and our understanding of the environment that nurtured early life on Earth is poor. Sophisticated isotopic analyses of Earth's oldest materials, including >4.0 billion year old zircon crystals and 3.9 to 2.5 billion year old sulfur minerals will be used by our team to determine the ages of Earth's atmosphere, hydrosphere, and geodynamo, relate these ages to the earliest signs of life, and assess how the emergence of life changed our planet. Experiments will be performed to investigate interactions between ancient microorganisms and their environs. Chemical interactions between organisms and their inorganic and organic environs will be monitored using the newly developed transition metal isotope systems. The pooled results from these disparate studies will provide new information about the antiquity of life on Earth and provide a firm basis for life-detection on other bodies in the Solar System.

The appearance of Eukaryotes in Earth history coincided with dramatic changes in the planet's climate, ocean chemistry, atmospheric chemistry, and tectonic configurations, suggesting that emergence of biological complexity may be linked to evolving climate. Understanding the early part of this progressive history may therefore reveal general principles that are applicable to the growth of complexity in any living system. UCLA lead team investigators are studying the evolution of eukaryotes using information from molecular biology and the fossil record. The goals are to better understand the order in which important universal properties of eukaryotes (nucleus, sterols, cytoskeleton, endoplasmic reticulum, organelles, multicellularity, etc.) were acquired, and to try to date these events using both the fossil record and molecular clocks. The nature of the common ancestor and the source of the prokaryotic donations to the genome(s) of the last common ancestor of all living eukaryotes are outstanding problems in evolutionary biology that will be addressed by our work. As part of this effort, CAB members will also examine the steps by which eukaryotes obtained and lost their energy-producing organelles.

The Cambrian period of Earth history was a time of rapid evolutionary innovation. The exponential advance in animal diversity and complexity near the start of the Cambrian demands understanding as it is one of the signature features in the evolution of life on our planet. Our group will examine this rapid advance by studying the

morphological development of trilobites, the emergence of skeletons by searching for the responsible developmental genes, and fossils that represent stem groups of modern phyla that reveal the ways in which animal body plans developed.

The UCLA Center for Astrobiology will continue to be engaged in numerous activities that serve to strengthen the field of astrobiology in general. These activities include continued support of the UCLA Astrobiology Society, introduction of an Astrobiology General Education course, participation in the NAI Minority Institution Involvement Faculty Sabbatical Program, participation in the Minority Institution Astrobiology Collaboratory (MIAC), organizing public lectures, and convening of Rubey Colloquia, which serve as forums for rapidly developing topics of interest. The Astrobiology Society is the first student-run organization devoted to fostering the discipline of astrobiology at the university level. In the coming years the Society will be engaged in replicating their success at other institutions, especially those serving minorities in particular. UCLA was one of the first hosts in the new Faculty Sabbatical Program and this connection has resulted in a closer tie between our lead team and the MIAC.

Executive Summary: University of Colorado Center for Astrobiology

The University of Colorado Center for Astrobiology continued its broad efforts that span the entire range of disciplines within astrobiology. We have substantial components within astrobiology research, teaching, and outreach, and each of these will be described briefly.

Within research, our efforts divide into several overall themes, focussing on the physical sciences, the biological sciences, and the humanities. Each of our nine Co-Investigators fits within one or two of these themes.

In the physical sciences, our efforts emphasize the formation of planetary systems (Co-I John Bally), the earliest environment and potential for life on the Earth (Steve Mojzsis), the nature and evolution of planetary habitability (Brian Toon), and the habitability and potential for life on Mars (Bruce Jakosky).

One of the outstanding questions in planet formation around newly forming stars is under what conditions planets will form and under what conditions they will not. Our new results involve observations in the Carina Nebula. This region is one in which abundant stars are forming. It was anticipated that the high density of massive stars would be destructive to the protoplanetary disks that eventually coalesce into planets. However, evidence for a large number of disks was found, making this nebula host to the largest population of disks outside of the Orion Nebula. Given that most low-mass stars are born in the immediate vicinity of highly destructive massive stars, either planet formation must be very rapid or exo-planets will be relatively rare.

Deciphering the history of the Earth's own habitability as a planet is central to understanding what makes a planet habitable. Our ongoing geological and geochemical approach to this problem included analysis of the so-called "mass-independent" sulfur isotope anomalies in Precambrian sediments. We have unequivocally demonstrated the existence of non-mass-dependent sulfur isotope fractionation in ancient sedimentary sulfides, using a new ion microprobe multicollector technique. This fractionation is thought to result from atmospheric effects involving various photochemical processes, so that its detection in sulfides tells us something fundamentally important about the nature of the atmosphere, the climate, and the oxidation state at the time of sulfide formation, as well as biogeochemical interactions in the sulfur cycle. This work is now being extended to the Great Oxygenation Event between 1.9 and 2.4 Ga ago.

We are applying our results to understanding the nature of the atmosphere, environment, climate, and habitability of Mars. Through a combination of laboratory studies and numerical modeling, we were able to show that carbon dioxide clouds are unlikely to have provided significant greenhouse warming on early Mars. This calls into question the greenhouse model that is the usual explanation for the early warm environment that allowed liquid water to flow (as reflected in the geomorphology of the surface). We also suggested an alternative model for carving the valley networks that involves transient liquid water at extremely high temperatures following impact events early in Martian history, rather than a steady-state greenhouse warming that would allow temperate liquid water to exist at the surface (Fig. 1). While we cannot yet choose between competing models, defining the range of models is important for understanding planetary habitability and our ability to extend our understanding of these processes to extrasolar planetary systems.

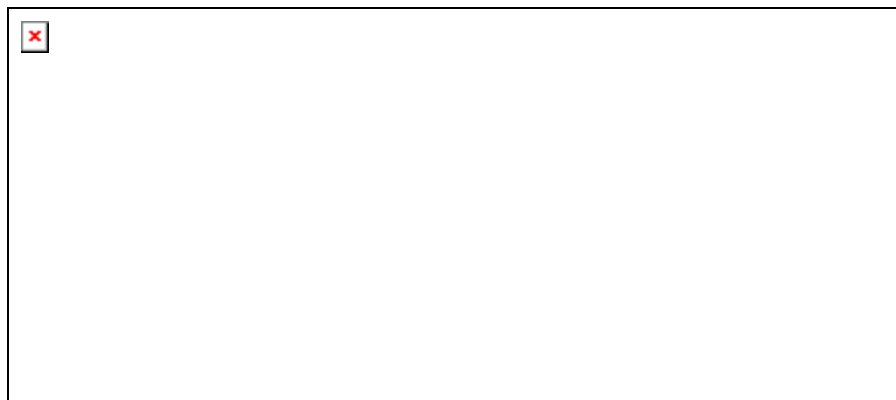


Figure 1. *This figure is a good summary of the work presented in the paper by T.L. Segura, O.B. Toon, A.*

Colaprete, and K. Zahnle *Science* 298, 1977-1980 on the environmental effects of large impacts on Mars. Panel A shows the amount of water from vaporization of the impactor (dotted curve), target material (dash-one dot curve), and polar caps (dash-two dot curve), from melting the subsurface (dashed curve) and the total (solid curve) as a function of impactor size. Panel B shows the time that the planet's regolith is above 273 K as a function of impactor size. The modeled objects (100-, 200-, 250- km diameter) are shown as data points.

Along the same lines, we are examining potential micro-environments on Mars in which liquid water could exist even at sub-zero temperatures. In particular, we are looking at the ability of thin films of liquid water to form at temperatures as high as -20°C in high-latitude ground ice or polar deposits of water ice. These temperatures can occur at moderate and high obliquity values, meaning that liquid water could have been accessible essentially at the surface as recently as a few million to a few tens of millions of years ago on Mars--essentially at the present epoch. Organisms can survive in these environments, so it is conceivable that evidence for Martian life could be found at relatively shallow depths below the surface (e.g., centimeters to meters, rather than hundreds of meters).

In the biological sciences, our efforts focus on the nature of the ribonucleic acid (RNA) World and the origin of life (Co-I Mike Yarus), gene duplication and the evolution of protein functions (Shelley Copley), microbial ecology and life in extreme environments (Norman Pace), and the evolution and nature of symbiosis (William Friedman).

What is the smallest useful RNA population? This question is key to understanding the accessibility of an RNA World, in that RNA is a very difficult molecule to make under primordial conditions. Therefore, the fewer molecules needed to provide for the evolution of biological functions, the easier it would be for them to occur; this is important both for understanding the origin of life on Earth and for understanding the potential for life to occur elsewhere. In the last year, we have shown theoretically in three different ways (two involving numerical calculations and one computer simulation) that the amount of RNA needed for evolution of an RNA cell (a "ribocyte") is likely to be ten or twelve orders of magnitude smaller than used in all modern selection experiments. Therefore, the RNA World seems to be immensely more accessible than has been previously supposed, and its existence as a real intermediate in the evolution of life on Earth seems much more likely. Of course, this previously unrealized 10^{10} - to 10^{12} -fold advantage must be tested experimentally.

We have begun a new project to probe the evolution of novel enzyme activities by the recruitment of pre-existing proteins. This will allow us to better understand the evolution of metabolic function in already-existing organisms and the evolution of life beyond the initial organism. While enzymes are highly evolved to perform certain functions, they often have advantageous secondary activities as a consequence of the assemblage of highly reactive groups in their active sites. If these secondary activities become useful to the organism, then the enzyme can be recruited to perform a new function, and subsequent mutations can then lead to improvements in that new function. This process has been profoundly important in the evolution of living organisms, and understanding the ability of organisms to move into new ecological niches requires an understanding of this process. We have begun an experimental program to explore this process. During this past year, we have generated a plasmid-based library containing all of the genes in *E. coli* that we will use to test the ability to recruit genes to replace ones that have been "knocked out".

A new direction involves "ecogenomics", the goal of which is to understand the organismal makeup of extremophile communities and how the individual kinds of organisms contribute to the overall concentration of biomass. We have been investigating hypersaline microbial mats, mainly at Guerrero Negro, Baja California. The results contribute to our knowledge of the diversity of life in extreme environments (Fig 2). Although substantial effort has gone into study of chemical aspects of the Guerrero Negro system, relatively little is known about the organisms that comprise these communities. Using molecular survey methods in which ribosomal RNA genes are obtained directly from the natural environment, our results show promise of revolutionizing our view of the makeup of such communities. For example, we find that cyanobacteria, while conspicuously present in these mats, are only one component, and generally a minor component, of the numerically dominant organisms. The generally more abundant organisms are representative of the "Green Nonsulfur" phylogenetic division of bacteria (Fig. 3). This was an unexpected result that changes fundamentally the way that the community needs to be modeled.

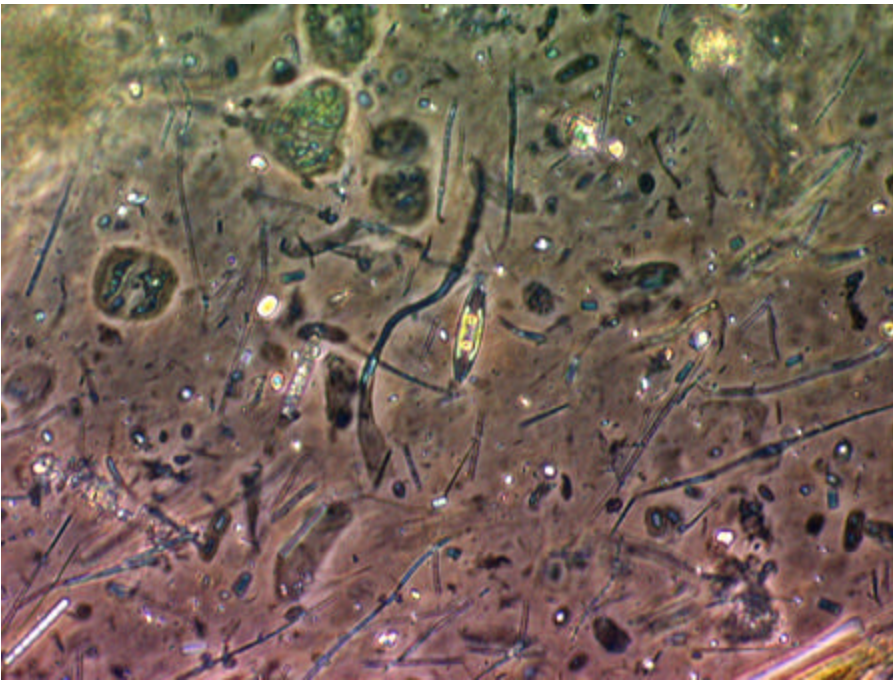


Figure 2. *Microbes in the hypersaline microbial mats of Guerrero Negro. CU Astrobiology Institute researchers, with other members of the NAI Ecogenomics Team, study these photosynthetic microbial mats as an example of extreme ecosystems. Molecular analyses have revealed that the genetic diversity of the communities is enormous.*

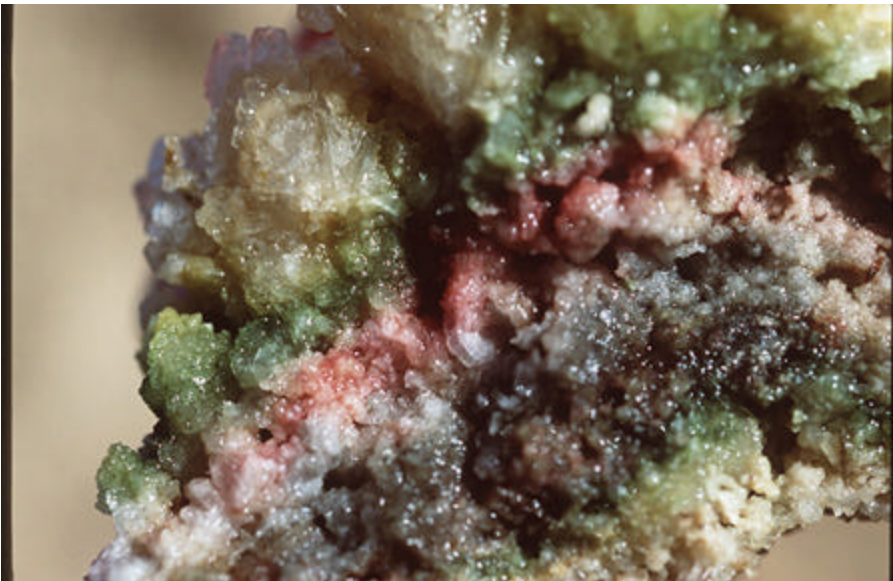


Figure 3. *Photosynthetic microbial communities in brine-saturated halite-gypsum crust from Guerrero Negro, Mexico. The Colorado Astrobiology Team studies these and other unusual microbial communities as models of life in extreme settings.*

Symbioses, the mutually beneficial relationships between two organisms, have evolved numerous times over the course of evolutionary history of life on Earth. Different forms of symbiosis have resulted in some of the most profound evolutionary radiations, such as the origin of the mitochondrion from a symbiosis between a proteobacterium and early eukaryotes, or the establishment of multicellular photosynthetic organisms (i.e., plants) from a symbiosis of fungus and green algae. We are characterizing the fungal symbiotic partners that may have been critical to the colonization of terrestrial (that is, land) environments by photosynthetic organisms. During the last year, we have discovered a complex relationship between early lineages of multicellular land plants and their fungal partners. During one part of their life cycle, the fungus provides nutrients to the host plant in exchange for a supply of fixed carbon; in another part, the symbiosis involves an entirely different fungal partner, and the plant is essentially a parasite on the fungus. This will allow us to determine whether plants radiated into terrestrial

environments alone or whether fungi in association with plants were the key to the single colonization of land by photosynthetic organisms. This will have important implications for the evolution of life both on Earth and potentially on other planets.

In the humanities, Co-I Carol Cleland works on the nature of astrobiology as a (predominantly) historical science and the definition of life. Her results on the nature of definition and the ability to define life are having an impact both nationally and internationally. They suggest that we do not have, at present, the ability to formulate a cogent definition for life, given the difficulty of determining which characteristics of life are specific to terrestrial life and which might be more generally applicable. In this context, attempts to define life are analogous to attempts to define "water" prior to the development of molecular theory--they just couldn't work.

In our teaching efforts, we offer both undergraduate and graduate courses in astrobiology, and we have recently instituted a graduate certificate in astrobiology that can be earned as an adjunct to a related graduate degree. Our mainstay undergraduate course is "Extraterrestrial Life", an upper-level non-majors course that provides an overview of the entire discipline of astrobiology; it regularly draws the maximum of 75 students each semester, and has been offered every semester for a half-dozen years. At the graduate level, we taught our graduate course in astrobiology; this course is geared toward providing a broad overview of the discipline for graduate students in either the physical or the biological sciences, and the course this year had about 15 students enrolled. In addition, a wide variety of more-specialized courses are taught within the individual disciplines of astrobiology; these include, for example, planetary atmospheres and surfaces, microbiology, and evolution.

Our outreach activities this past year involved two main efforts. First, we continued our very successful series of public symposia with one entitled "Life on Earth--and Elsewhere?" This forum provided an opportunity in our fifth year of NAI funding to spotlight our own program, with several of the Co-Is presenting their research results across the entire spectrum of topics. Second, we began what we hope will be a long series of outreach symposia in which we take the excitement of astrobiology to other institutions. We sponsored a symposium on "Life in the Universe" at Ft. Lewis College in southern Colorado, with presentations by several of our Co-Is. This allowed us to talk about cutting-edge research and exciting directions to students who don't regularly have a chance to see this type of work, and to interact with both students and faculty in informal settings. Our intent was to use this as a "tune up", and now to begin a regular series of taking the program to other institutions at no cost to them. We have plans for a second symposium, and will be taking it to a variety of institutions that are attended in large part by students from backgrounds that are not well represented in the sciences.

In summary, we have a vigorous and first-rate astrobiology program at the University of Colorado, and we are making major progress in many of the different areas of astrobiology. Through our Center for Astrobiology, we can bring all of the components together into a broader understanding of life on Earth and the potential for life to exist elsewhere.

The Origin, History, and Distribution of Water and its Relation to Life in the Universe

Intellectual Focus & Proposed Research --

Water is the medium in which the chemistry of all life on Earth takes place. Water is the habitat in which life first emerged and in which all of it still thrives. Water has modified Earth's geology and climate to a degree that has allowed life to persist to the present epoch. We propose to create a research and education framework that links the biological, chemical, geological, and astronomical sciences to better understand the origin, history, distribution, and role of water as it relates to life in the universe. We focus on scenarios involving the sources and distribution of water in planetary systems and the delivery and incorporation of water into rocky planets that orbit within the "habitable zones" of their parent stars. Our framework will include and connect research on major aspects of planetary water – in effect we aim to understand the terms of a "watery Drake equation":

- ✦ Observations and modeling of the abundance and distribution of water in the interstellar medium, molecular clouds, and circumstellar disks;
- ✦ Surveys and studies of the numbers, water content, D/H ratio and dynamics of icy outer solar system bodies such as comets, Centaurs and Kuiper Belt objects;
- ✦ Laboratory experiments on complex molecule trapping and formation on interstellar water-rich grain mantles and planetary ices;
- ✦ Cosmochemical studies of meteorites that record the incorporation of water into silicate material in the primordial Solar System as an early step in its eventual inclusion in larger bodies, including planets;
- ✦ Models of the escape of water (as hydrogen) from the atmospheres of Earth-sized planets;
- ✦ Spacecraft- and meteorite-based research and theoretical studies on the role of water in forming the diversity of rocks and sediments on Earth, Mars, and Venus.
- ✦ Biological exploration of ice-covered habitats in Iceland, Antarctica, and North America with potential application to the search for life on Mars and Europa.
- ✦ Biological and chemical exploration of extreme aquatic habitats in and around the Hawaiian islands including the deep-sea, Kauhako Lake crater on Molokai and Lake Waiau near the summit of Mauna Kea.
- ✦ Development of concepts and prototype hardware for instruments that could be used to detect and characterize life on other planetary bodies.

Distinguishing Features –

By developing and testing models and exploring the outcomes of alternative scenarios we seek to determine what controls the abundance and distribution of water and hypothetical aqueous habitats in other planetary systems. This research will directly support the NASA search for past or present life on Mars and efforts (such as the NASA Terrestrial Planet Finder) to directly detect and characterize Earth-sized exoplanets. The proposed framework of our research will support a group of postdoctoral scholars who will carry out independent, interdisciplinary research spanning two or more of the investigators' (or affiliated investigators') research specialties. The island setting and the unique facilities of the University of Hawaii offer an environment conducive to these investigations. The University of Hawaii houses world-class research groups active in astronomy, and in the chemical, geological and biological sciences. It is home to a substantial array of scientific facilities, including the telescopes of Mauna Kea Observatory, a fleet of research vessels for oceanographic and deep-sea investigations and facilities for exploration of the extreme environments found on the volcanoes and in the lakes and oceans around Hawaii. Through this Astrobiology proposal we aim to combine and capitalize on these many areas of research excellence to craft a new, interdisciplinary study of water and its relation to life.

Management Approach –

The UH-NAI PI will meet once a week with the Co-Investigators to discuss research progress, discuss any technical, financial or management issues, and to involve everyone in the E/PO activities. The timelines and status of the projects will be reviewed and updated at these meetings. In addition the UH-NAI will offer an astrobiology seminar series which will involve the entire local community. Additionally the development of our collaborative visualization tool will help foster interdisciplinary communications and highlight areas of new development which can be discussed at these regular meetings.

Education/Public Outreach Activities –

Our group has a strong record in education and public outreach, and we intend to build on that experience by providing innovative laboratory-based learning opportunities for teachers and engaging the public in our research. We intend to develop a program that trains teachers in the science of astrobiology, how to incorporate astrobiology into their classes, and in how to use the activities we will develop as part of the program. The focal point of our teacher-training program will be a summer professional development program. Using the summer workshop and research experience as a base, we will also develop a course for pre-service teachers. All projects will involve development of standards-based classroom activities. We also intend to actively engage the public in our discoveries.

Institutional Commitments –

The University of Hawaii and the Institute for Astronomy are strongly committed to the success of this project, and have committed a total of \$4.5 million in faculty positions, in-kind salary support and cash contributions to the project.

Collaborative Networking Concepts –

We will design, implement and evaluate a software visualization tool to interface with the NAI Science Organizer knowledge management tool.

Executive Summary: University of Rhode Island (URI)

The University of Rhode Island (URI) team of the NASA Astrobiology Institute (NAI) works to gain a fundamental understanding of Earth's subsurface life. The team's research is principally, but not exclusively, focused on study of life in deeply buried marine sediments. Earth's deep biosphere is a critical component of Earth's biogeochemical cycles and serves as a model for possible life on other planets. Consequently, the team's objectives are to understand the subsurface microbial ecosystems of marine sediments, their role in Earth's biogeochemical cycles, and their relevance to the search for life on other planets.

Environments of special interest for our team include: 1) old, deeply buried sediments where life exists despite extremely low concentrations of electron donors and key nutrients, and 2) hot, deeply buried anoxic sediments where life may exist independently of the photosynthesis-based ecosystem at Earth's surface. The ecosystems of these subsurface habitats are potentially representative of the ecosystems that may exist on other planets.

The team's investigators are based at the University of Rhode Island, Woods Hole Oceanographic Institution, and the University of North Carolina at Chapel Hill. To effectively accomplish their objectives, URI team researchers collaborate with each other and with scientists at other institutions throughout the world. For example, over the last year, team members have collaborated closely with scientists at the Massachusetts Institute of Technology, the Max Planck Institute for Marine Microbiology (Germany), the NASA Ames Research Center, Pennsylvania State University, the University of Aarhus (Denmark), and the University of Miami. To gain access to subsurface environments, URI team members participate in international expeditions through the Ocean Drilling Program (ODP).

Over the last decade and a half, scientists throughout the world have documented the widespread occurrence of life deep beneath Earth's surface. However, fundamental questions about Earth's subsurface life have yet to be answered. The URI team is actively addressing many of these questions: What categories of organisms live beneath Earth's surface? How are they related to surface organisms? How closely related are subsurface organisms in geographically distant but similar environments? How much living biomass inhabits Earth's subsurface environments? What do subsurface organisms do for a living? How active is subsurface life? What is its effect on the surface world?

To explore the taxonomic composition of subseafloor ecosystems, URI team members recently finished a 16S ribosomal ribonucleic acid (rRNA) survey of bacteria and archaea in subseafloor sediments of the Nankai Trough (Kormas et al., 2003) and are working on genetic studies of subseafloor sediments from ODP Leg 201 (in the Peru Margin and the eastern equatorial Pacific). Karen Lloyd, Mark Lever and Andreas Teske are working to isolate, polymerase chain reaction (PCR) amplify, clone and sequence diagnostic key genes for methanogens and sulfate reducers from Leg 201 samples. Antje Lauer, Ketil Soerenson and Teske are developing deoxyribonucleic acid (DNA) isolation procedures and documenting bacterial and archaeal 16S rRNA profiles from the Leg 201 samples, using Denaturing Gradient Gel Electrophoresis (DGGE).

Our genetic studies to date have revealed high diversity in subseafloor prokaryotic communities e.g., Kormas et al, 2003). They have also shown that distinct communities occur at different subseafloor depths. Our studies of bacterial nucleic acids demonstrate that several unique bacterial lineages occur repeatedly in deep subseafloor sediments, suggesting that deep subseafloor sediments constitute a unique environment (Kormas et al, 2003). These unique lineages are candidates for sharing physiological characteristics that predispose them to a subsurface mode of life. Our studies have also identified a specific archaeal phylotype (within the genus *Thermococcus*) with a trans-Pacific distribution pattern (Figure 1) (Kormas et al., 2003). This archaeal distribution and the repeated occurrence of bacterial lineages mentioned above demonstrate the "connectedness" of life across great distances in deep subseafloor sediments.

URI team members have also made significant headway toward documenting biogeochemical signatures of present and past microbial processes. Kai-Uwe Hinrichs, Helen Sturt, Kristin Smith and Roger Summons' analyses of intact polar lipids from microbial isolates demonstrate great potential to distinguish microbial phylotypes in natural samples on a species level. John Hayes, Hinrichs and Laura Hmelo are actively developing protocols for isotopic analysis of volatile fatty acids in subseafloor pore waters. Hinrichs, Wolfgang Bach, Hmelo and Sturt are undertaking mechanistic studies of biological hydrocarbon formation in the deep subsurface (Hinrichs et al., 2003).

These Year-5 biosignature studies have led to several interesting results. For example, one line of our biosignature study showed that concentrations of intact polar lipids in the deep subsurface are generally orders of magnitude lower than in surface sediments, reflective of significantly lower densities of microbial populations. Another line of study suggests that ethane and propane are biologically created by hydrogenation of small carbon-bearing organic and inorganic molecules in a variety of deep subseafloor sedimentary environments. Finally, a third line of biosignature study demonstrated that adsorption of biologically produced hydrocarbons is an important process in subseafloor environments. By removing these hydrocarbons, this adsorption enhances free energy yields and allows biological production of the hydrocarbons to continue in subseafloor environments (Hinrichs et al., 2003).

URI team members are also advancing understanding of subseafloor microbial activity and its effects on Earth's biogeochemical cycles. To assess relationships between cell counts and community activity, David C. Smith, Beverly Chen and Andrew Staroscik developed protocols for adenosine triphosphate (ATP) analysis of subseafloor sediments and completed ATP analyses of ODP Site 1230. Scott Rutherford, Steven D'Hondt and Arthur J. Spivack mapped global patterns of subseafloor sulfate reduction (Figure 2) and estimated their effect on the global sulfur cycle (Rutherford et al., 2003). Guizhi Wang, Rutherford, D'Hondt and Spivack are using chemical data and biogeochemical flux models to estimate net rates of several metabolic processes in subseafloor sediments (D'Hondt et al., 2003; Wang et al., 2003).

The early results of our ATP studies suggest that the active subseafloor biomass is substantially smaller than estimates of subseafloor biomass based on cell counts. This finding suggests that the living subsurface biomass may be substantially lower than generally estimated (the dead may greatly outnumber the quick in subseafloor body counts). Despite this low estimate of living biomass, our studies of subseafloor sulfur fluxes indicate that prokaryotes in deeply buried marine sediments play an important role in the global sulfur cycle; our global flux calculations indicate that subseafloor activities permanently remove about 50% of the sulfur that enters the ocean each year (Rutherford et al., 2003).

Graduate, undergraduate and post-doctoral education and research are integral parts of the URI team's mission. During the Year-5 report interval, our active research team included four post-doctoral scholars (Lauer, Soerenson, Sturt and Rutherford), three graduate students (Lever, Lloyd, and Wang) and five undergraduate students. All of these individuals played vital roles in our ongoing program. To introduce astrobiology to young scientists with particularly strong potential, this year the URI team initiated nationally competed summer research fellowships for upper-class (junior or senior) students to work with URI investigators on projects of astrobiological significance. Two summer fellows (Uri Manor and Beverly Chen) were selected from 16 applicants in this year's competition. Chen is working on the ATP studies mentioned above. Manor is working with Rutherford, Wang and D'Hondt to develop an inverse-modeling program for estimating net rates of microbial activity from deep subsurface biogeochemical data. Both students are expected to complete their fellowships in the Year-5 report period.

To build a deeper institutional base of astrobiology awareness among undergraduate and graduate scientists and engineers, URI astrobiologists expanded the academic programs of their home institutions by integrating astrobiological themes into existing microbiology courses, as well as by offering a new URI graduate course, "Subsurface Life" (Autumn, 2002), and an upper-class undergraduate URI engineering and science course, "Exploring the Ocean of Europa" (Spring, 2003). To introduce our work and the field of astrobiology to the broader public, URI team investigators gave public presentations and lectures at a variety of universities and other venues. To disseminate our work and its relevance more broadly, in the Year-5 interval the team also completed and now maintains a website on URI astrobiology efforts in research and education (<http://www.gso.uri.edu/astrobiology>).

In short, research, education and outreach by the NAI URI Team and its collaborators are steadily advancing knowledge and awareness of life deep beneath Earth's surface, its role in Earth's surface processes, and its relevance to the search for life on other planets.

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Executive Summary: University of Washington

Our research at the University of Washington has centered on three important astrobiological questions: What are the characteristics of planets that can evolve complex organisms? Where might such planets occur? How does biological complexity evolve on a planet, and how might it end?

We are beginning the third year of research into these questions. Below, our results and progress is summarized based on specific research problems defined in our original proposal.

How often, where, and under which conditions do habitable planets form and persist?

We define a habitable planet as a solid body capable of supporting life as we know it. The study of extra-solar habitable planets involves a broad interdisciplinary approach that extends from understanding how planets are formed to understanding the conditions that allow such life to originate, survive, and evolve.

During the past year (2002-2003), Lucio Mayer and Tom Quinn Mayer and Quinn have continued performing simulations of giant planet formation by fragmentation of a gaseous disk, and have published their results in Science. They are extending previous work by considering the effect of different equations of state on the outcome, and by continuing the calculation for longer periods of time. Lufkin and Quinn have started simulations of giant planet migration in gaseous disks, while Barnes and Quinn in collaboration with Lissauer (Ames) continue their simulations of planetesimal accretion in the terrestrial region. They have made progress in speeding up this computationally challenging calculation. The aim is to determine the mass spectrum of planetesimals coming out of the middle stage of planet formation as a function of distance from the Sun. The information to date suggests that the gravitational instability model appears to be viable, and therefore giant planets should be common. Secondly, planet migration appears to be a chaotic process.

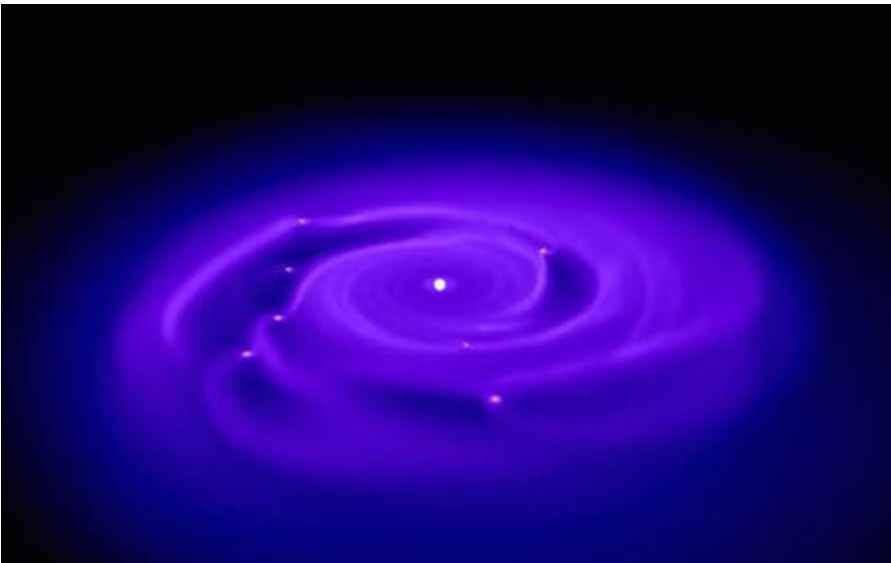


Figure 1. Gasdynamical simulations of planet formation show that giant planets can be formed in hundreds of years. Mayer et al, 2002, Science.

Our team is also examining metallicity gradients in nearby stars. These new observations of recently announced stars with planets continue to confirm their previously reported high metallicities relative to otherwise similar field stars. We have also uncovered preliminary evidence that giant planets being discovered by the Doppler method are less common around K and M dwarfs than they are around G dwarfs.

What caused the Delivery and retention of organics and volatiles through Earth History?

The volatile and organic composition of impacting bodies is a key factor in the evolution of habitable planets. What are the relative roles of large comet and asteroid fragments and interplanetary dust particles (IDPs) in bringing these materials to a planet? In collaboration with George Cody of Carnegie NAI lead team, Brownlee and Kress

have found that organic material found in bulk meteorites can be released into the gas phase when subjected to atmospheric entry conditions (flash-heating at a rate ~500 K/sec and peak temperature of 1000 K for a few seconds). These compounds may have played important roles in the atmospheric chemistry of early Earth, during a time when the flux of micrometeorites was much higher than today. In particular, all of the compounds named above are greenhouse gases. We also found numerous small polycyclic aromatic hydrocarbons, which would have been excellent absorbers of ultraviolet (UV) radiation during a time when no ozone layer existed. Over 250 200µm diameter unmelted micrometeorites were identified and extracted from sediments recovered from the unique South Pole Water Well sample provided by collaborator Susan Taylor from CRREL in Hannover. Graciela Matrajt (an astrobio-supported Post Doc visiting from France) extracted the particles, crushed them and treated them with HF. AIB was discovered in these samples, the first amino acid detection in micrometeorites. We are studying all three of these processes and their effects on prebiotic evolution.

How do mass extinctions and impacts affect the evolution and survival of complex organisms, i.e., the long-term habitability of planets?

Mass extinctions are short-term events that kill off a significant proportion of a planet's biota, and on Earth have been of greatest consequence to more complex organisms such as metazoans. Surface life is vulnerable to major planetary catastrophes, for example, impact of a large comet or asteroid, radiation and particles from a nearby supernova, or catastrophic climate changes such as intense intervals of greenhouse heating or Snowball-Earth type episodes. It is now known that at least one of the major mass extinctions was caused by large body impact, and we would very much like to know if others as well are related to this. The major line of study involves the study of impact craters and their history. Impact cratering involves evaluation of projectiles, which to first order is a measure of the asteroids and comets passing in near Earth space. More broadly it involves the origin of those objects, the orbital evolution of the time of their existence, and at least in the case of asteroids, a series of collision and fragmentation processes that occurred in the asteroid belt. Once the materials are in near Earth space, it involves the evaluation of the material passing through atmospheres of various densities (and in some cases not passing through the atmosphere, and then the mechanism of the hypervelocity impact event itself. The physics of the impact include the explosive release of the kinetic energy, which produces the vaporization and melting of rocks and excavation of material not only as an ejecta blanket in the immediate vicinity of the crater, but in the case of large impact events (which can be biologically significant) the ejection of debris through the atmosphere into space where it can then envelope the entire planet. The biological consequence for the sudden release of this energy can occur on many scales from affecting individuals to the death of species and even removal of entire ecosystems or biota. The mechanics, biological and geological consequences of impact cratering is an enormous field in the very sweep of its questions.

During the past year, two major mass extinction boundaries were studied: the Permian-Triassic (P/T) boundary in Africa and Canada, and the Triassic-Jurassic (T/J) boundary in the Queen Charlotte Islands, Nevada, Italy, and the Newark Basin. Isotopic and paleontological results from these sites are now either in progress or are finished. Our new work suggests that neither the P/T nor T/J mass extinctions were caused by large body impact.



Figure 2. Co-I David Kring on the Triassic/Jurassic boundary, New York Canyon, Nevada. Photo by Peter Ward

What can we learn from the geological and fossil record about the evolution of eukaryotes and metazoans?

Though we can extrapolate back from existing organisms or build theoretical biogeochemical models, the only robust empirical data that show how early life on Earth evolved and interacted with its environment comes from the study of early Precambrian rocks. Despite popular misconceptions, globally there is in fact a moderate abundance of well-preserved Archean (>2.5 Ga) and Paleoproterozoic (2.5 -1.6 Ga) rocks, which can serve as our clearest windows on the events that occurred shortly after life's origin. Further research was performed in 2003 on Archean hydrocarbon biomarker geochemistry, sulfur isotopic fractionation, and Paleoproterozoic hydrocarbon preservation in fluid inclusions. Also, new research was initiated in the areas of metamorphism of Archean biosignatures, Archean paleobarometry, and on the age and origin of controversial "microfossils" putatively of Archean age from the Pilbara Craton, Australia. Field-work was conducted on Hadean supracrustal rocks from Isua, Greenland, the early Archean Apex Basalt near Marble Bar, Australia, and the late Archean Fortescue Group near Tom Price, Australia. This research has shown that cyanobacterial and eukaryotic lipids are present in rocks half a billion years before other fossils of these groups appear in the geologic record and that molecular fossils can survive for much longer under higher thermal regimes than previously expected.

Also, we discovered that microbial sulfate reduction existed in ~3.5 billion year old oceans and this reduction shows that peripherally branching bacterial phyla had already evolved. Finally, and perhaps most significantly, the work of our group, based on mapping of the ~3.5 billion year old Apex Basalt, indicates that the "microfossils" previously reported from this unit are not as old as the surrounding rocks.

What can we learn from the physiology and molecular characteristics of extant life about the evolutionary pathways by which microbes and their communities evolve, and by which complex organisms originate?

The 0.5 Byr period prior to 3.5 Ga probably experienced extensive evolutionary experimentation and very limited physiological diversity. Recent evidence also points to lateral gene transfer between Bacteria and Archaea as the main mechanism involved in the formation of eukaryotes. Subsequently, symbioses between specific kinds of bacteria and eukaryotes contributed to the rise of oxygen-respiring and oxygen-producing multicellular biota. Today, both lateral gene transfer and interdependence of different species living in communities are ancient processes that continue in the microbial world.

The invention of multicellularity was a major biological innovation contributing to new states of biocomplexity. This was preceded, however, by the invention of eukaryotic cellular organization. The genome sequences from Bacteria have yielded many surprises, including the presence of genes thought only to be present in eukaryotes. We have

found genes for tubulin in four species of *Prostheco bacter*, a genus of the bacterial division, Verrucomicrobia. The discovery of tubulin in bacteria raises questions as to their origin. They could have been transferred horizontally from a eukaryotic organism to these bacteria or vice versa. The goal of our current research is to better understand the origin of the bacterial tubulin genes that are very distantly related to those from eukaryotes, indicating that the transfer, if it occurred, must have happened a long time ago. Based upon partial genome sequences that are available, we are currently trying to determine which of the three hypotheses seems most reasonable. The most readily tested hypothesis is the last one. If it is correct, then we would expect that a substantial number of eukaryotic genes would be found in the genomes of the Verrucomicrobia. Preliminary results based upon Blast searches of genes of the Verrucomicrobia indicate, however, that fewer other eukaryotic genes have been found in the *Prostheco bacter* genome than expected based on the simplest variant of this model, that the Verrucomicrobia are the founding member of the Eucarya.

What was the nature of early Earth communities?

A second way to examine the ancient Earth is through study of microbial communities that likely resemble those of the Precambrian. These communities include those found in (a) anaerobic and photosynthetic microbial mats and biofilms, (b) the sub-seafloor associated with deep-sea hydrothermal vents, and (c) water ice. It is possible that hidden in the presently unknown diversity of these ecosystems there exist organisms with metabolic pathways that are relics of common metabolisms of the past. We participated in one Early Microbial Ecosystem Research Group (EMERG) field trip in 9/2002 to the Guerrero Negro evaporation ponds to study microbial mat populations in these ponds. These mat populations are visually homogeneous over kilometers of extent and display steep geochemical gradients (e.g. light, oxygen) with depth. We are assessing bacterial diversity using Terminal Restriction Fragment Length Polymorphism (TRFLP), a rapid method for determining total community structure and composition. We have assessed diversity over two scales: horizontally, at varying spatial scales over a kilometer distance, and vertically, using cores sliced at submillimetric scales with depth. Results indicate that these bacterial communities are remarkably stable across even large spatial scales (~1 km); however some variation even at fine scales (cm) was detected. Highly significant differences have been seen with depth in the mat over the upper 9 mm of the mat and significant differences between day and night samples were detected, suggesting significant migration of microorganisms may be occurring.

The second project is investigating a specific functional assemblage of microorganisms, the sulfate-respiring prokaryotes (SRP). High levels of activity for this group have been measured in this site. A wide diversity of SRP has been identified and significant differences in SRP community composition with depth were revealed. Significant correlations of SRP community structure with geochemical gradients were detected.

What is organismal metabolism in extreme environments?

Another approach to understanding the habitability of planets is to consider the range of extreme environmental conditions on Earth that support life. For example, the detection of water ice and/or submarine hydrothermal vent systems on another planetary body would satisfy some of the key criteria for habitability. Laboratory observations of bacterial motility and field observations of bacterial attachment led to the testable hypothesis that a temperature threshold exists in ice formations (-10 °C in sea ice), below which bacteria cease moving as a means to locate optimal resources and conditions and instead become attached to a surface (first-stage biofilm formation), allowing for continued activity down to -20 °C (lowest temperature tested yet). We have also introduced a new aspect to first-stage biofilm formation in the cold: the role of bacterial viruses in altering bacterial behavior and possibly triggering attachment. Building on our finding of considerable morphological diversity of bacteriophage in subzero Arctic seawater and on field work last fall (CASES 2002 expedition), novel phage-host systems were established in the laboratory, using the obligately psychrophilic (cold-loving) bacterium, *Colwellia psychrerythraea* strain 34H (whole genome sequence available), as the host. Initial experiments have revealed a potential link between phage infection of a bacterium and its production of exopolymers that promote attachment.

Towards Characterization of Extrasolar Terrestrial Planets

"There are countless suns and countless earths all rotating around their suns in exactly the same way as the seven planets of our system. We see only the suns because they are the largest bodies and are luminous, but their planets remain invisible to us because they are smaller and non-luminous. The countless worlds in the universe are no worse and no less inhabited than our Earth."

- GIORDANO BRUNO (1584)

Are we alone? The richness and enormous vastness of our universe makes our solitariness seem almost unthinkable, and so this ancient musing has remained one of the most fundamental of human questions. Yet while the medieval monk Giordano Bruno could only speculate on the prevalence of other planetary systems that could harbor life, in the last decade we have been fortunate enough to see the search for other worlds evolve beyond the realms of heresy and science fiction, into science theory, and finally, into science fact.

As this report is being written, we now know of 107 planets outside our own solar system. These planets have been discovered using existing ground-based telescopes. However, in the coming decades NASA and the European Space Agency (ESA) will design, build and fly space-borne observatories to expand our knowledge of the number and distribution of planets in our Galaxy, and to determine whether or not these planets might support life. These missions, such as NASA's Kepler, Terrestrial Planet Finder (TPF), and Life Finder will specifically search for habitable terrestrial worlds, that is, Earth-sized rocky worlds that can maintain liquid water on their surfaces for extended periods of time. TPF, planned for launch in 2015, will be designed to directly detect Earth-sized worlds around nearby stars, and to determine whether or not these sister planets are habitable, like our own Earth, and will initiate the search for the global signs of non-technologically advanced life. The most ambitious of these planned missions, Life Finder, is envisioned to build upon the discoveries of TPF, to provide a more detailed spectroscopic search for non-technologically advanced life on planets in our Solar neighborhood.

To optimize the designs and search strategies for these NASA missions, and to ultimately interpret the data that they return, we must expand our ability to recognize worlds that might have habitable conditions, and to discriminate between planets with and without life, based only on remote-sensing observations.

Characterizing Extrasolar Terrestrial Planets

Methods for characterizing terrestrial planets in our solar system have been refined for decades by planetary and Earth-observing scientists. Some of these techniques can be adapted for use in the characterization of terrestrial planets around other stars. Examples include time-resolved whole-disk photometry, spectroscopic remote sensing for the detection and retrieval of atmospheric and surface composition and physical parameters, and time-resolved spectroscopy of spectral features to look for diurnal or seasonal variations in surface albedo or atmospheric composition. However, since existing observing techniques are not yet sensitive enough to directly detect and gather information on Earth-sized planets around other stars, our principal means of advancing our understanding of these planets is via theoretical modeling studies. So, to improve our understanding of the potential range of environmental and spectroscopic characteristics for terrestrial planets in our galaxy, our team is developing a suite of innovative modeling tools to simulate the environments and spectra of a range of plausible extrasolar planets and for the early Earth.

These modeling tools comprise a Virtual Planetary Laboratory (VPL). This tool will incorporate models to couple the radiative fluxes, climate, geology and biology of a terrestrial planet to produce a self-consistent state for a broad range of candidate planetary environments. Self-consistency between a planet's chemical, physical, and biological properties state is particularly important when trying to understand the detectability of biosignatures in the spectrum of the planet's atmosphere and surface. This effort is strongly interdisciplinary, calling upon the expertise of planetary scientists, astronomers, biologists, geologists, chemists, mathematicians, computer scientists and statisticians to all work together towards a focused common goal. This effort therefore exemplifies the science of astrobiology, an interdisciplinary approach to understanding life in our universe.

The Virtual Planetary Laboratory

The core of the VPL is a coupled radiative transfer/climate/chemistry model, which is being assembled from existing models that have already been validated individually and used to address many key scientific problems in planetary and Earth sciences. This coupled-climate-chemistry model is being augmented by interchangeable modules currently under development. These modules consist of geological, exogenic, atmospheric escape, and life process models that will be used to characterize fluxes of heat and trace species at the upper and lower boundaries of a planetary atmosphere.

The VPL will be validated using data derived from terrestrial planets in our own solar system. It will then be used to explore the plausible range of atmospheric compositions and thermal structures, and to generate disk-averaged spectra for extrasolar planets and for early Earth. These models will be run with and without biological processes to improve our understanding of the effects of life on a planet's atmospheric composition and spectrum. They will also be used to create a spectral catalog that can be used as a statistical sample space to explore the optimum wavelength range, spectral resolution, and instrument sensitivity required to characterize extrasolar terrestrial planets.

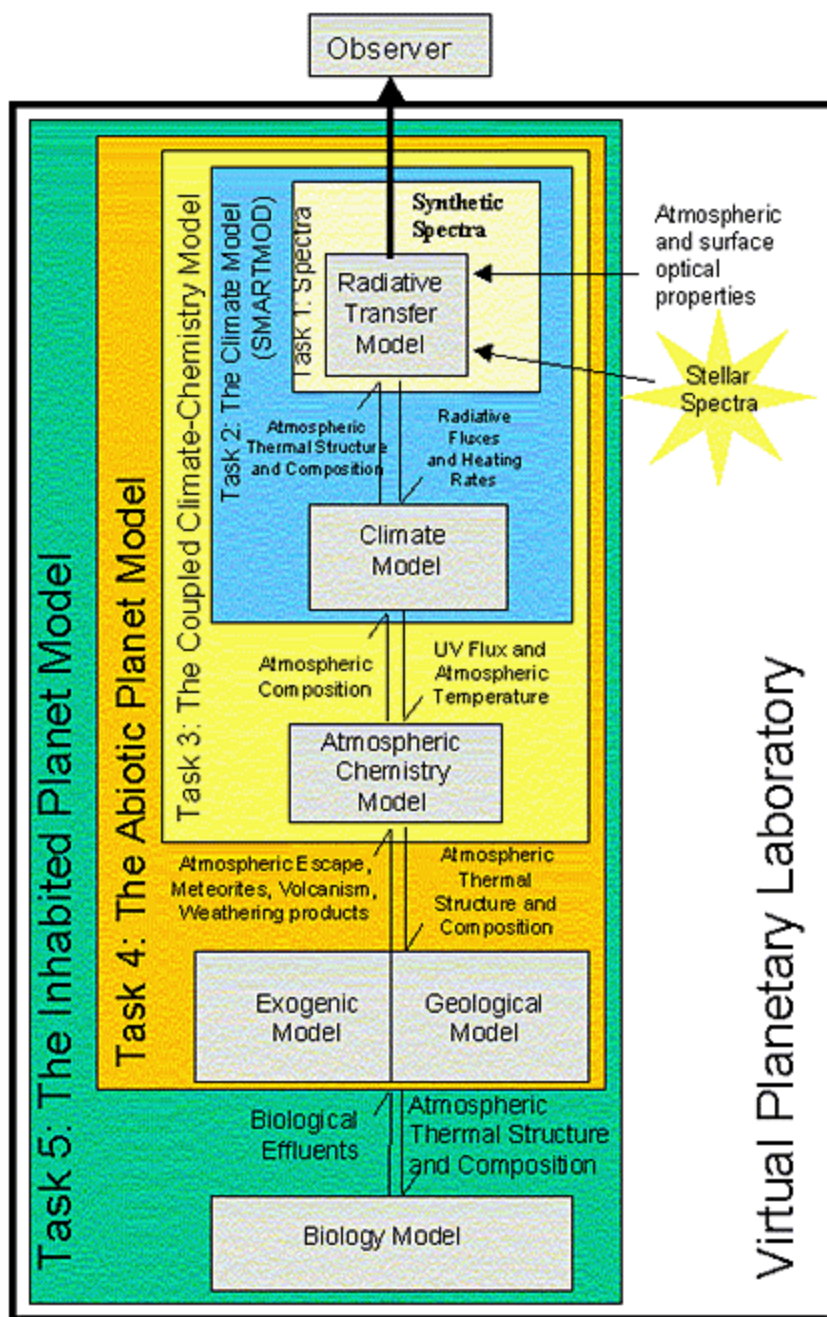


Figure 1. The suite of radiative transfer, climate, chemical, geological, and biological component models are shown as boxes, and their interactions with each other are shown as arrows. The information transferred between these component models is labeled at each interface. The order in which these component models are coupled to each other during the course of building the VPL is specified by the Task number. The radiative transfer, climate and atmospheric chemistry models already exist, and the remaining models are under development by our team. At each task development stage the model can be used to generate synthetic spectra to derive required capabilities for astronomical instrumentation.

Progress This Year

Assembling the Virtual Planetary Laboratory

In the second year of this program, we continued our long-term efforts to develop the suite of VPL models. Focusing initially on the architecture of the integration, we defined the basic structure and communication modes for the integrated model. This included defining the requirements for the model-to-model translators and the nature and

content of the common database that will hold initialization information, model input and output, and control status flags. Integration of the radiative transfer and climate models is completed, with the climate and chemistry models to follow. The geological, exogenic and biological processes will initially be specified as "state vectors" in the common database, to allow testing on the larger model, and later will be replaced with the full analytical or numerical models.

We have updated our one-dimensional (1-D) radiative-convective equilibrium (RCE) climate model to better support interaction with the geological and life components of the planet. We upgraded the RCE model to incorporate our line-by-line, multiple scattering radiative transfer model in place of simpler band modeling and 2-stream methods. A mixing length approach has been implemented to provide a self-consistent description of the vertical transport of heat and volatiles throughout the environment. Both these new features enhance the model's ability to provide climate constraints needed for the geological and life processes. We also added a simple cloud model for formation and dissipation of clouds, which will later include precipitation.

Work has also proceeded on defining the "boundary layer" geology and atmospheric escape models. We have planned a series of 1-D reactive transport models to characterize biogeochemical cycling in different environments, which will couple to the atmosphere and lithosphere via flux terms fed through the database. This design draws upon team work performed this year on modeling to analyze Archean paleosol isotopic measurements. This work has improved understanding of elemental cycling on ancient Earth and will provide empirical constraints for VPL models of the Archean Earth.

This year, team members also worked on geology models that link the planetary boundary layer with the planet's mantle and core. This included the development of physical models to reconcile geochemical observations, and to understand the link between plate tectonics, thermal evolution and degassing for the Earth throughout its history. Team members have also explored the link between planetary thermal evolution and the generation of the planetary magnetic fields that can strongly affect long-term planetary habitability.

Looking to the upper planetary boundary layer, we have developed a new technique to model hydrodynamic loss processes from planetary atmospheres that overcomes previous difficulties and instabilities in modeling supersonic outflow. We have validated a preliminary model, which will be incorporated as an upper boundary module for the VPL. This model can be applied to both terrestrial planets and extrasolar gas giant planets.

To support the VPL life modules, we have conducted both field work and modelling studies. We have continued our field assessments of life in high-pH aquifers and springs associated with ultramafic rocks, an environment analagous to early Earth's, or an undifferentiated water-rich terrestrial planet. Our objectives are to understand how microbial communities may have fared in primordial environments without prior chemical weathering, to understand nutrient limitations, and to look for biosignatures associated with these communities. Several organisms have been isolated, and a baseline for annual and seasonal variations in geochemical conditions is emerging.

We have also been developing an anaerobic early Archean ecosystem model to understand biological productivity and methane production prior to the origin of oxygenic photosynthesis. The model explicitly accounts for cycling of hydrogen and carbon through primary production by hydrogen utilizing methanogens, fermentation, methanogenesis and photolysis. The ecosystem model has been run in conjunction with an atmospheric chemistry model. Initial results indicate that for a volcanic flux of hydrogen at the modern rate, the atmosphere could contain up to ~350 ppmv of methane if hydrogen utilizing methanogens were the primary producers. This level of methane would produce a strong signature in the spectrum of the planet.

We have also initiated a dynamical model of a microbial ecosystem, which includes interaction with the external environment, including the stellar flux. In this multicomponent model, individual microbial species interact with each other, and their growth is governed by available energy and chemical resources, and limited by space. We continue to explore the stability of these equations, for both the autonomous and environment-coupled system. We are also working on expansion of the model to include stellar-driven photosynthetic life.

In addition to development of the architecture and individual models, we are also gathering spectroscopic information required as input for extrasolar planet models. Both as ongoing support to the planetary modeling efforts, and as part of independent research to explore the concept of the "G star analog" we have combined astronomical observations and photospheric models to create high-resolution, full-wavelength range spectra for

stars of different spectral type. Team members have also collated an ongoing, yet already comprehensive, database of spectral line lists and absorption cross-sections for 45 molecules required by planetary atmosphere models.

In addition to initiating the assembly of the VPL itself, our team has also worked on two related proof-of-concept or precursor projects, both of which use component models of the VPL to explore the detectability of biosignatures via remote-sensing techniques. These modeling efforts and results are described below.

Spatially Resolved Planetary Models

The first such project uses the VPL's radiative transfer model to produce spatially resolved spectral models of planets in our own solar system. The 3-D "datacube" of synthetic spectra that is generated for each planet is being used to determine the effects of spatial and spectral averaging and temporal variability on the detectability of biosignatures, and other spectral features that provide important constraints on a planet's physical and chemical state, and its potential for habitability. These models are also being created as a validation standard to test progressive development of the larger VPL model. This year, we completed the Mars model, producing disk-averaged synthetic spectra and lightcurves, and used this model and TPF instrument models to understand our ability to detect the CO₂ ice cap in the disk-averaged spectrum of a successively more frozen planetary surface.

We are currently working on the comparable Earth model. As an experimental validation component for this project, our collaborators at the Australian Center for Astrobiology (Bailey and Chamberlain) continue to acquire and analyze high-resolution full-disk spectra of Mars and Venus using ground-based telescopes.

Earths Around Other Stars

In collaboration with the Penn State NAI team, we have explored the environments and detectability of biosignatures from extrasolar terrestrial planets similar to the Earth. Using a coupled 1-D radiative-convective climate model, we have determined atmospheric compositions for Earth-like planets with oxygen levels from the present atmospheric level (PAL), down to 1/100,000th PAL, around stars hotter and cooler than our own Sun. We have also run self-consistent models for atmospheric conditions during the Earth's early Proterozoic. We have used the VPL radiative transfer model, and the detailed spectrum of the parent star, to generate high-resolution synthetic spectra for these atmospheres, which we have analyzed for detectability using TPF instrument simulator models. These spectra are the first steps in building a spectral library of terrestrial planets for use in TPF mission planning and data analysis.

Significant results included an improved understanding of the behaviour and detectability of ozone and other atmospheric constituents for an Earth-like atmosphere at different oxygen levels. These simulations have also provided significant insight into the combined effects of temperature and trace gas distributions on the detectability of biosignatures, especially around stars of different spectral type, and the results are directly relevant to the Terrestrial Planet Finder mission. As part of this project, we also calculated surface ultraviolet (UV) fluxes and UV dose rates for erythema and deoxyribonucleic acid (DNA) damage. We find that planetary UV surface fluxes are low for high-oxygen planets orbiting the high-UV output F stars, because of the creation of a protective "super" ozone layer. These results will help us to further understand what effects stellar type and planetary atmospheric composition might have on planetary habitability and biological evolution.

Extrasolar Hot Jupiters

We have developed a 1-D chemical model for extrasolar "hot Jupiters" for which the formation of hydrocarbons, oxygen chemistry, and hydrodynamical loss are significantly enhanced because of their environment. Significant results include an improved understanding of the chemical mechanism for H and H₂ production in these unusual planets. The modifications to the chemical model to incorporate new chemistry, database modeling, and to couple it to a hydrodynamic escape code are directly applicable to modifications required for the VPL terrestrial planet models.

Education and Public Outreach

Our EPO team this year has participated in planning meetings for the Cosmic Origins travelling museum exhibit and is developing a framework for a family guide on Life Elsewhere. The EPO team has also run workshops on

astrobiology and astronomy at several conferences. Scientific team members have participated in or contributed to many courses, presentations, talks and media interviews, including 7 university courses, 19 university seminars, a talk to middle school students, 7 talks at museums or observatories, and 6 interactions with the media, including interviews for public television. Team scientists have also participated in preparing and reviewing EPO material for the web. Further information about the VPL can be found on our public website, which was launched this year: <http://vpl.ipac.caltech.edu>.

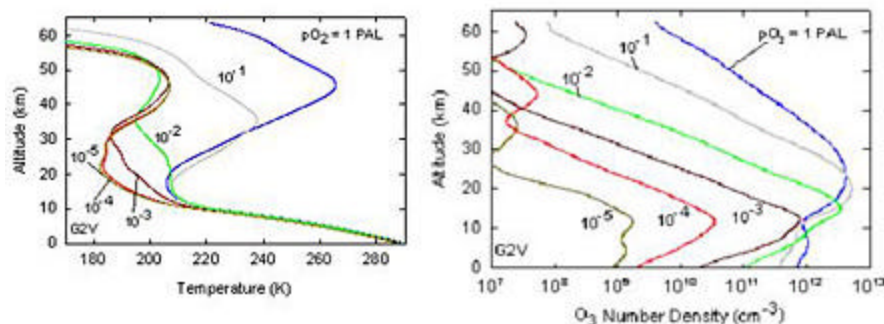


Figure 2. Plots showing a) atmospheric temperature and b) ozone mixing ratio, as function of altitude for Earth-like planets with successively lower abundances of oxygen in their atmospheres. The blue line shows results for an atmosphere with the present atmospheric level of O₂, and the other lines are labeled with the factor of 10 reduction in oxygen abundance from the present level. These plots show the gradual cooling of the stratosphere, and the descent of the peak concentration of ozone in the atmosphere as a function of the decreasing atmospheric O₂ abundance.

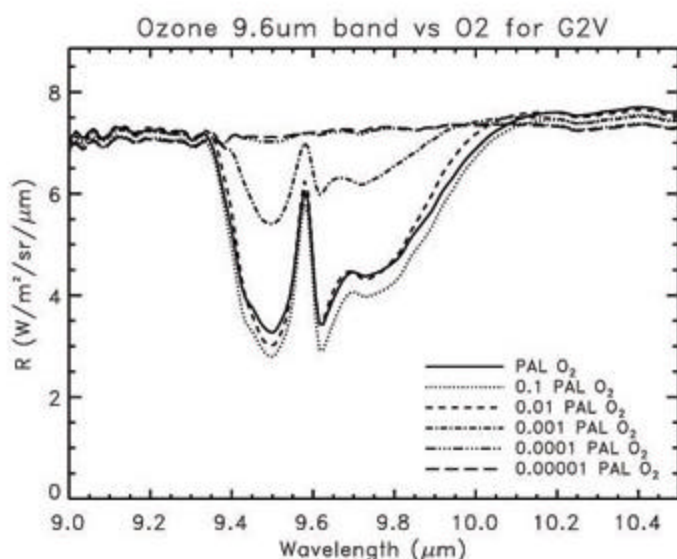


Figure 3. This plot shows the ozone band as a function of atmospheric oxygen abundance for a planet around a G2V star. The ozone column decreases, but remains appreciable down to O₂ levels of 0.01 PAL, but the 9.6μm ozone band retains almost the same strength down to this O₂ level because the lower ozone column is offset by enhanced absorption due to the greater temperature difference between the stratosphere and the warm, emitting planetary surface.

Executive Summary: Astrobiology Society of Britain

Director/Leader: Charles Cockell

Introduction

The Astrobiology Society of Britain emerged in 2003 from the UK Astrobiology Forum and Network. In an effort to formalize the network, which was previously an informal e-mail list of those in the UK interested in Astrobiology, the society was established. The Society maintains affiliate status with the NAI and is the professional society representing the astrobiology community in the UK. Its membership is international in scope and it has members from the US and European countries. The main purpose of the Society is to organize meetings and aid discussion amongst astrobiologists in the UK and to involve itself in international efforts such as fund raising for international fellowships.

Recent developments

UK Astrobiology Conference

In March 2003, the UK held its first astrobiology meeting in Girton College, Cambridge. The meeting was split into two days. The first day was a general meeting covering many aspects of astrobiology from the origins of life to robotic astrobiology. It was an inclusive day, with discussion encouraged amongst any members of the community with interests in astrobiology who wished to attend. The second day was a more specialist day, focusing on the conditions for the origin and emergence of life. Over 80 people attended the conference, making the meeting a great success. UK corporations with an interest in astrobiology also attended the event.

Astrobiology Society of Britain

During the Girton meeting it was proposed that the UK astrobiology forum and network be disbanded and replaced by a new society which would more formally represent the interests of the UK astrobiology community. Whereas previously the forum was an unelected informal committee, the committee of the new society would be an elected, accountable structure. Over 50 people paid an initial membership fee of £5.00 to join the society. Following the meeting elections were held for the committee and the society was established.

Other developments

The Society comes into being during an exciting time for the UK in astrobiology. The UK-led exobiology lander, the Beagle 2, is on its way to Mars and is expected to begin its work on the surface of Mars in December 2003. The Open University held its inaugural opening of its Center for Astrobiology early in the year, demonstrating the interest in astrobiology amongst UK universities. Other meetings with astrobiology themes have been held in the UK, for example the Society for General Microbiology held a special session on astrobiology. Within this context, the environment for the existence of the Society is good.

Future developments

The Society will hold its first committee meeting in September 2003, where the direction and future plans of the society will be discussed. Possible directions of the Society include:

- Planning another UK astrobiology meeting
- Raising funds with the Federation of Astrobiology Organizations (FAO) for an international fellowship.
- Promoting astrobiology by co-sponsoring meetings with other organizations
- Expanding its membership

General publications

Astrobiology 2003. Abstracts of the UK Astrobiology Meeting, Girton College, Cambridge
(www.astrobiologysociety.org.uk)

Executive Summary: Groupement de Recherche en Exobiologie

Director/Leader: François Raulin

GDR Exobio

GDR CNRS 1877, or Groupement de Recherche en Exobiologie is a national federation of research laboratories in Exo/Astrobiology, created by the National Centre of Scientific Research (CNRS) in 1999 and renewed in 2003. Its main goal is to promote, induce and coordinate the development of Exo/astrobiology scientific programs in France. It includes about 50 laboratories, and is supported by the CNRS and CNES (the French Space Agency).

4 main research topics are addressed:

=> The ingredients of primitive life in their geological, planetological and interstellar context

=> From the chemistry of the origins of life to the emergence of life: structures and functions of biological molecules

=> Terrestrial life as a reference: fossils, biomarkers, extreme environments

=> Habitats and signatures of extraterrestrial life

GDR Exobio has a scientific Council consisting of 19 scientists from different fields, including astrophysics, planetology, physics, chemistry, earth sciences, life sciences, as well as philosophy and epistemology. The GDR has been affiliated with the NAI since November 2001.

Main recent activities

During the period July 2002-June 2003, the GDR Exobio, within the framework of its main 4 topics, has been active in several directions directly connected to NAI activities. Below are just a few examples of these activities, with the corresponding references given in the Reference list.

Several important results on Titan's organic chemistry (Raulin & Owen 2002) have been obtained by joint studies involving GDR and other teams, including the determination of optical properties of Titan's tholins (Ramirez et al., 2002) and the potential presence of oxirane on Titan (Coll et al, 2003). Evidence that POM may be a precursor of formaldehyde in comets has been obtained through laboratory experimental data and modeling (Cottin et al, 2003). The development of methodologies and instrumentation for in situ exobiologically oriented analyses of Martian soil has been reported by GDR & US teams (Buch et al., 2003; Cabane et al, 2003).

The effects of solar radiation on amino acids and their derivatives exposed in Earth orbit has been examined (Barbier et al., 2002a,b; Boillot et al., 2002). The effects of interstellar and atmospheric mechanisms on the prebiotic synthesis have been studied (Meierhenrich et al., 2002). The identification of stable, biogenic, chiral aliphatic hydrocarbons by GCMS was demonstrated by Meierhenrich et al. (2003). An experiment simulating artificial meteorites showed that extraterrestrial sediments can reach the Earth (Brack et al., 2002a,b). Evidence for the fractionation of oxygen isotopes during entry of artificial meteorites into the Earth's atmosphere has been shown (Miller et al., 2002).

The environment of the early Earth as the context for early life on Earth and as an analogue for life on other planets has been the subject of a number of publications (Westall et al., 2002; Westall et al., 2003; Westall and Drake, 2003; Westall, 2003a,b). Modern and ancient biofilms from the Early Archaean have been studied as analogues for potential extraterrestrial biofilms (Westall and Walsh, 2003; Walsh and Westall, 2003; Toporski et al., 2003a,b). It has been shown that previous investigations for microfossils and for a biogenic carbon isotope signal in the Early Archaean, 3.7 to 3.8 Ga rocks from Greenland did not take into account the presence of modern contaminating microorganisms (Westall and Folk, 2003). The evolutionary status of the earliest microfossils from 3.3-3.5 Ga rocks from Barberton and the Pilbara was examined by Westall (2003c). Stromatolitic microfossils preserved by hematite in the 2 Ga Gunflint Formation serve as an analogue for potential Martian microfossils in the Meridiani Planum hematite deposit (Allen et al., 2003).

The presence of various microbial eukaryotes in deep-sea hydrothermal settings and the association of potential biogenic signatures to sulfur-metabolizing bacteria have been shown (López-García, 2003a, 2003b).

Our efforts were also devoted to investigate one of the most important biological aspects of the origins of life, that is the RNA world hypothesis (Meli et al, J. Biol. Chem, 2002; 2003). On the other hand, we were able to show by

Surface Enhanced Raman Spectroscopy (SERS) the detection of subpicomolar amount of nucleic acid components trapped on mineral surfaces (Elamri et al, in press).

The search for indications of biological activity on extrasolar planets is also an important part of the studies which are carried out by the GDR team which involve large-scale cooperation, especially with other teams linked to the NAI (DesMarais et al, 2003).

Exobio'03, the 3rd bi-annual National exo/astrobiology summer school will take place in Corsica in September 2003. As for the 2 previous schools, it will be attended by close to 100 participants coming from astronomy and geophysics (60 persons) and from chemistry and biology. The program of this year includes: chronology: comparison of time scales in astronomy, geophysics, chemistry and biology; impacts and Earth bio-geo evolution; Moon formation; chemical and biological evolution; auto-organisation and complexification; exoplanets; life on Mars, Titan, Europa; interstellar chemistry; LUCA; Artificial life.

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Future Hopes

The GDR wishes to participate in cooperative programs of research, on clearly identified topics, in the framework of its affiliation to NAI. The NAI connection should be a great opportunity to share and exchange researchers, in particular PhD students, to promote joint participation in research programs on Exo/astrobiology, and to use the synergy induced by the different ways of thinking and working of researchers from different origins to promote the wide field of Exo/astrobiology. Targets such as Titan, in relation to Cassini-Huygens, Mars and its international program of exploration, Europa and Extrasolar planets are already among the topics of high interest likely to be part of such joint research projects. In addition, the need for networking, with new and efficient tools for teleconferencing (including asynchronous communication) remains a technical priorities.

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Executive Summary: European Exo/Astrobiology Network Association (EANA)

Director/Leader: André Brack

Introduction

The European Exo/Astrobiology Network Association, EANA, was created to co-ordinate the different European centres of excellence in exo/astrobiology or related fields already organised in national networks. The specific objectives of EANA are to bring together European researchers interested in exo/astrobiology programmes and to foster their cooperation, to attract young scientists to this quickly evolving interdisciplinary field of research, to interface the Network with European bodies such as ESA, ESF, the European Commission and with non European institutions active in the field and to popularise exo/astrobiology to the public and to students. The EANA includes presently 12 European nations active in the field: Austria, Belgium, Denmark, France, Germany, Italy, Portugal, Spain, Sweden, Switzerland, The Netherlands, and United Kingdom.

EANA is affiliated to the NASA Astrobiology Institute. The formal affiliation was signed in September 2002 at the Graz Workshop by Rosalind Grymes, Deputy Director of NAI, during a reception hosted by the Governor of Styria in the historical Eggenberg Castle

Recent activities

(The activities of associate CAB, and affiliate GDR Exobio and UKAN will be reported separately)

Second European Workshop on Exo/Astrobiology

The Second European Workshop on Exo/Astrobiology was held in Graz, Austria, 16-19 September 2002, co-organized by EANA and the University of Graz and the Institute of Geophysics, Astrophysics and Meteorology. Attended by 320 participants, the workshop was oriented towards planetology. The scientific input of the Graz meeting was published as ESA SP-518.

European Commission COST Action

Within the framework of EC COST Actions to foster co-operation in a specific research area, the First COST D27 Workshop "Origin of life and early evolution" was held in Ravello, Italy, 17-20 October 2002. Approximately 70 participants took part in what was a very successful "kickoff" activity of the group. Sessions were organized on prebiotic chemistry and chirality, astrobiology and early molecules, RNA, cooperativity, self-replication, autocatalysis, and prelude to the Cell. The proceedings of the workshop will be published as a special issue of Origins of Life and Evolution of the Biosphere.

ISSI, International Space Science Institute activities in Astrobiology

The International Space Science Team "Prebiotic matter in space" is a consortium of 12 scientists, each representing a specific research field crucial to revealing the origin of life as a consequence of the evolving Universe. The ISSI Astrobiology team organized a workshop at the International Space Science Institute in Bern, Switzerland between April 1-4, 2003 on the topic "Astrobiology - Future perspectives". The workshop was restricted to 35 top experts. The meeting results will be published in a book by Kluwer Academic Publisher in spring 2004.

EGS-AGU-EUG Joint Assembly in Nice

Several sessions at the European Geophysical Society - American Geophysical Union - European Union of Geosciences (EGS-AGU-EUG) Joint Assembly in Nice, France, from April 6 - 11, 2003, dealt with astrobiological topics. The session "Life on the fringe: biotic survival over geological time scales and in subglacial environments" was attended by about 75 participants. A special issue of "Astrobiology", will feature several key note papers. The session "Water and life" was also very successfully attended by more than 70 participants. Many of the papers presented will be published in a Special Issue of the International Journal of Astrobiology.

Darwin/TPF and the search for extrasolar terrestrial planets

The conference, held in Heidelberg, Germany, 22-25 April 2003, was attended by about 200 participants. The topics covered included: Results of Planet Searches, New Search Strategies, Formation and Evolution of Planetary Systems, and Conditions for Life. Proceedings are currently in press in the ESA-SP series.

Beagle 2

Beagle-2, the UK led exobiology lander of ESA 2003 Mars Express mission, comprises an integrated suite of instruments to optimize the search for evidence of life on Mars in subsurface and rock interior samples. The package includes instruments to study sample mineralogy (composition, macroscopic and microscopic structural and textural features), organics (elemental, molecular), oxidation state and petrology (major and minor element composition). Environmental characteristics will also be measured (atmosphere, radiation budget, temperature, pressure, wind speed and direction, humidity, dust activity). Launched on June 2, 2003, Beagle 2 will be operating on Mars in December 2003.

Future activities and objectives

The Third European Exo/Astrobiology Workshop will be hosted by the Centro de Astrobiologia in Madrid, November 18-20, 2003. The Workshop will be dedicated to the search for life on Mars.

The following objectives will be pursued:

- ✍ to enlarge EANA to other European countries active in Exo/Astrobiology, i.e. Finland, Hungary, Poland, Romania, and Russia
- ✍ to interface the Network with European bodies such as ESA, ESF, the European Commission and with non European institutions active in the field
- ✍ to promote the Federation of Astrobiology Organizations, FAO
- ✍ to popularise Exo/Astrobiology to the public and to students

General publications

- ✍ Exo/Astrobiology, Proceedings Second European Workshop on Exo/Astrobiology, Graz 2002, Ed. H. Lacoste, ESA Special Publication ESA SP-518 (2002). "Astrophysical and Astrochemical Insights into the Origin of Life", Reports on Progress in Physics 65, 1427-1487 (2002), co-authored by the the ISSI Team members.

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